ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTES OF HEALTH

1960

NATIONAL HEART INSTITUTE

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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REPORT OF 1960 NHI PROGRAM ACTIVITIES
REFERENCE SHEET

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PHS-NIH
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Administration
(Organization)

The National Heart Institute, under the National Heart Act, is charged with the responsibility for: the conduct of research relating to the causes, prevention, and methods of diagnosis and treatment of diseases of the heart and circulation; assisting and promoting such research by other public and private agencies; coordinating all research results and promoting their application; providing training as necessary; and assisting local and State agencies.

The accomplishments of the Heart Institute administrative offices, from the standpoint of both program direction and administrative services provided, are most properly reflected in the achievements of the component units of the Institute: The Grants and Training Branch; the Intramural Research Branch; the Biometrics Research Branch; the Heart Information Center; the Geographic Pathology Section; the Field Epidemiological Research Section; the Framingham Heart Disease Epidemiology Study; and the Heart Disease Control Program (organizationally located in the Bureau of State Services). Contributions to the research program by these units are described elsewhere in this report.

The administrative function is constantly being re-evaluated in terms of how well it assists the operating people to do their jobs adequately. Following is a list of the changes and developments in administrative areas during the year 1960.

1. Dr. James Watt, Director of the National Heart Institute, will return full-time to his NHI duties after completing his assignment as Special Assistant to the Secretary for Aging during the past year. Mr. Robert H. Grant, Executive Officer, NHI, who served as Director, Special Staff on Aging and Staff Director of the White House Conference on Aging during 1960, has accepted a position with the Office of International Research Activities under the Office of the Director, NIH.

2. Implementation of Mr. John Reed's report and recommendations on the budget process in NHI has resulted in the development of improved methods for reporting to program officers.

3. In order to reduce the scientific and administrative time involved in writing individual position descriptions on fairly standardized technician positions, the Personnel Generalist assigned to NHI has written and established standard descriptions for GS-2 and GS-3 positions to be used throughout NHI. In addition, she has initiated the establishment of a standard description for GS-5 Chemist positions in the NHI research area.

4. Approval has been requested for organizational changes within the NHI. These changes involve the following: Establish the Cardiology Branch; Elevate the Experimental Therapeutics Section of the General Medicine and Experimental Therapeutics Branch to Branch level; Elevate the Clinical Endocrinology Section of the General Medicine and Experimental Therapeutics Branch to Branch level; Transfer responsibility for clinical evaluation of new techniques and instruments from the General Medicine and Experimental Therapeutics Branch to the Clinical Director; Abolish the General Medicine and Experimental Therapeutics Branch. It is believed that the proposed changes will result in more productive use of personnel in research and patient-care areas.

5. Dr. Donald Fredrickson, Section on Metabolism of the Laboratory of Cellular Physiology and Metabolism has been selected as Clinical Director of the Intramural Research Program and will assume these responsibilities during the coming year.

6. The Biometrics Research Branch has been established from the Biometrics Research Section and additional professional statistical personnel have been appointed to carry out expanded programs.

7. The Geographic Pathology Section, formerly administered in cooperation with Dr. Thomas Dublin's group in NIAMD, has moved to space in the Perpetual Building in Bethesda and the two programs separated administratively.

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HEART INFORMATION CENTER

The Heart Information Center carries out a program of public and professional information relating to the heart and circulatory system in health and disease, as authorized in the National Heart Act.

The diversified activities of the Center include the preparation and distribution of information-education publications, such as leaflets, pamphlets, and other items; issuance of releases and providing assistance to the press, periodicals, and science writers; preparation of reports, summaries of research findings, and other special materials; planning and development of exhibits, audio-visuals, graphics, and materials for broadcasting; maintenance of an inquiry and reference service; and cooperation with other agencies and organizations in information-education matters relating to heart and blood vessel diseases.

With specific reference to research in cardiovascular disease, the Center prepares and distributes information concerning research developments and advances, thus assisting the dissemination of new knowledge and helping to further the use of research findings. The Center also provides assistance to the research staff of the Heart Institute by furnishing consultation and services as needed in matters involving media of communication, including press relations, preparation of exhibits and other graphics, and clearance and reproduction procedures.

Materials produced and activities conducted by the Heart Information Center during 1960 are briefly summarized below.

EXHIBITS. Two new exhibits were produced in 1960, one for scientific meetings and one for the general public and public health groups; and one existing exhibit was modified. In addition, three exhibits constructed during previous years were widely scheduled and used.

"Directions of Present Day Research in Gerontology," a scientific exhibit, was executed by HIC for the Gerontology Branch in Baltimore. First shown at the International Association of Gerontology in San Francisco, it was later displayed at the Maryland Conference on Aging, and prominently featured at the Second White House Conference on Aging in Washington, D. C.

Designed for the general public and public health groups, "Stop Rheumatic Heart Disease Before It Starts," was exhibited four times during 1960---and at the last showing of the year, at the American Public Health Association meeting in San Francisco, it was awarded a Certificate of Merit by the Association's Committee on Exhibits.

An existing NHI-AHA exhibit was considerably modified and then shown at the National Conference on Social Welfare in Atlantic City under the new title "Working Together: National Heart Institute-American Heart Association."

Scheduling and staffing exhibits constructed during previous years continued to be an important function of HIC. Three of these exhibits, "Cellular Physiology of Aging," "Understanding the Heart and Circulation---From the Discovery of the Circulation to Modern Cardiology," and "Transseptal Left Heart Catheterization" were shown to a total of seven new audiences during the year.

Opportunities for cooperative exhibit projects were also numerous. As the result of one of these projects a HIC staff member was assigned full-time to the Division of International Health for approximately four months. There he assisted in the production of a special U. S. exhibit for the first International Health Fair in Rome, a major exhibition that invited health displays from 70 countries.

PUBLICATIONS. During 1960 the Heart Information Center issued two new publications, edited and reproduced two, reprinted four others, and prepared one for production and issuance by a non-government organization.

The fourth annual compilation of NHI grants, entitled Public Health Support of Cardiovascular Research, Training, and Community Programs, was printed and distributed. Highlights of Heart Progress---1959, a selection of items of interest from research studies conducted and supported by the National Heart Institute, was published. HIC edited and reproduced the proceedings of the National Advisory Heart Council Alumni meeting and also, Notes on Current Research in Cardiovascular Disease in connection with the Fifth Postgraduate Course of the American College of Physicians given at the Heart Institute. The four reprinted publications were: 1) A Living Pump, 2) Cerebral Vascular Disease and Strokes, 3) Rheumatic Heart Disease, and 4) Diet, Hormones, and Atherosclerosis.

At the request of the National Health Education Committee, Inc., a non-profit organization, the Heart Information Center wrote copy and prepared artwork for a compilation of selected research accomplishments of recent years, indicating the progress made against heart disease.

REPORTS. Preparation of regular and special reports in whole or in part was an important continuing function. Such reports included the regular weekly report containing items of interest on important research accomplishments and program developments for information of the Director, NIH, the Surgeon General, PHS, and others; the annual report of the Heart Institute for publication in the full DHEW annual report; and an annual report on information operations.

Among special reports prepared were brief summary statements for the Secretary of DHEW on cardiovascular surgery, rheumatic fever control, and heart drugs; material outlining subject fields of NHI-conducted and supported research and research reporting practices, for use by the National Sciences Foundation in connection with a publication being prepared on NIH; a resume

of NHI information activities for the Conference of State and Territorial Directors of Health Education; and a report for the White House (Dr. Snyder) summarizing developments of the past few years in four areas of cardiovascular investigation -- atherosclerosis, drugs for hypertension, lipid research, and anticlotting agents.

The Heart Information Center also participated extensively in development of materials required in connection with budgetary and appropriations hearings. Documents prepared in whole or in part included the Director's opening statementfor hearings on the Institute's fiscal 1961 appropriation; a compilation of highlights of research progress made in NHI-conducted and grant-supported investigations during the 1960 calendar year, together with selected highlights in program development which occurred during the year; a special-status report on congenital heart disease and one on primate research centers for submittal during the hearings; and other requested material.

PRESS RELEASES, RADIO and TV. Seventeen press releases or non-technical abstracts for press use were issued during the year. Nine of these reported on research findings and developments in Heart Institute laboratories, three announced appointments to membership on the National Advisory Heart Council, one announced an Institute staff appointment, and one disseminated information on a course of instruction to be presented at a professional meeting by an NHI staff member. The other three released information on the awarding of grants for a Regional Subhuman Primate Center, the visit of a Soviet delegation to the Heart Institute to participate in plans for a U.S.-U.S.S.R. program of scientific cooperation in the field of heart disease, and the joint statement by the American Heart Association and the National Heart Institute urging routine vaccination against influenza for persons with heart and blood vessel disease.

Ten of the releases involved cooperation with other organizations. Six were released at the annual meeting of the Federation of American Societies for Experimental Biology, and one each at meetings of the American Association for the Advancement of Science, the American Chemical Society, and the International Academy of Pathology. The statement on influenza vaccination was issued simultaneously by the National Heart Institute and the American Heart Association.

In the media of radio and TV, materials prepared included a script for interview of the Director, NHI, on the NBC program Monitor; a script outline for an hour-long TV program on Heart Disease Diagnosis, one of a series planned for telecasting in the Washington, D. C. area; and material for use in 5-minute TV film interviews of Director, NHI by Senator Smathers of Florida, on the subjects of Heart Disease and Aging. The film "Report to the Nation" was made available for general use by deposit of prints with the film service of the Communicable Disease Center and listing it for free loan in the Public Health Service Film Catalogue issued by CDC.

SERVICES TO SCIENCE WRITERS. Background material on cardiovascular disease subjects was furnished, special information was obtained and provided, and interviews with NHI scientists and staff members were arranged for a

number of science writers, reporters, and others. In addition to furnishing requested information and material, photographs were supplied or arrangements made for taking photographs in several instances.

Among publications serviced during the year were Business Week, Time, Newsweek, Medical News, Scope Weekly, Science News Letter, Chemical and Engineering News, Wall Street Journal, and Drug Research Reports. Cooperation was also given to science writers of various news services and organizations, including the Associated Press, United Press International, Newspaper Enterprise Association, Science Service, Scripps-Howard, World Wide Medical News, Voice of America, United States Information Agency, those on the staffs of some large metropolitan papers, and on the public relations staffs of a few medical schools.

SPEECHES AND ARTICLES. The Heart Information Center wrote or edited eight speeches and introductions, wrote four articles for non-government publications, submitted assorted pieces to the NIH Record, and composed two short messages for The White House.

Three of the eight talks were introductory remarks: Dr. Shannon's introduction of Dr. Homer W. Smith at the 10th Annual NIH Lecture, Dr. Terry's introduction of Dr. Scheele at the Fall meeting of the Association of Military Surgeons, and Dr. Watt's introductory remarks at the Conference on Prosthetic Valves for Heart Surgery. One talk was a complete text: "Problems of Heart Disease," delivered by Dr. John Miller of Heart Disease Control Program at a regional meeting of the American Heart Association.

Three speeches were edited for publication in 1960: "The Role and Support of Research in the Advancement of Medicine," a speech by Dr. Watt, for the Isidore W. Held Lecture publication; "Research and the Federal Government," a speech delivered by Dr. Watt, for Group Practice, an A.A.M.C. journal; and "Cardiovascular Research in the Public Health Service," a speech given by the Surgeon General, for The American Journal of Cardiology. A fourth speech, one delivered by Dr. Watt, was edited for inclusion in Exercise and Fitness, a collection of papers presented at the Colloquium on Exercise and Fitness.

Two articles on heart disease were prepared for encyclopedias, one for the Funk and Wagnall's Encyclopedia and one for the World Scope Encyclopedia. An article, written for Dr. Watt's byline, appeared as the lead article in the Irvington House 10th Anniversary Souvenir Journal. A fourth article entitled "The National Heart Institute," also for Dr. Watt's byline, was featured in Life and Health.

Numerous items were prepared for the NIH Record, including a feature piece on gas chromatography. A Presidential message, written in this office, was read at the American College of Cardiology; and a message for Mrs. Eisenhower was read at the 40th anniversary of Irvington House.

INQUIRY AND REFERENCE SERVICES. Numerous requests for information from the general public, individuals in medical and health professions, and private and public agencies and organizations, were received and expeditiously

handled during the year. Many indirect inquiries, referred from other units of PHS and DHEW, other government agencies, and the Congress, were also answered. The majority of informational requests were effectively met by furnishing pertinent leaflets or other appropriate publications; others required special compilation of information or material, consultation with scientists, or preparation of reading or reference lists.

The reprint reference collection maintained by the Center, containing papers on the research findings resulting from NHI-conducted and grant-aided investigations, continued to prove increasingly useful in connection with information activities, particularly research reporting. The up-to-date bibliographic information on Institute-connected papers appearing in technical journals, which is compiled and recorded for reference use in card files, has been very helpful to the Center. This information is also now being furnished on a routine basis to the Grants and Training Branch of the Institute.

SPECIALIZED INFORMATION PROGRAMS. The Heart Information Center carried out or provided assistance on a variety of special projects and programs, each tailored to meet the needs and interests of a specific audience. Individualized programs were developed during the year for groups of physicians, nurses, educators, science students, and others. Such projects included a three-day program involving three Institutes for five physicians who were founding the Marshfield Clinic Foundation for Medical Research and Education; a program on heart research for 42 professors of health and physical education from universities and colleges throughout the country; a special program for 60 educational supervisors from the State of Georgia; and one for orientation of 30 new medical officers of the Heart Disease Control Program. Among others for whom special programs were conducted were visiting representatives from the New Jersey Department of Health, Delaware State Board of Health and Heart Association, the New York Heart Association, and the Arlington (Va.) County Health Department.

The Center also provided assistance in connection with several meetings, including a one-week postgraduate course conducted for the American College of Physicians by the National Heart Institute which was attended by 165 physicians; the meeting at NHI of four Soviet physicians with officials and consultants of the Heart Institute to plan a joint program of cooperation in the field of heart disease; the Annual Cardiac Symposium for physicians sponsored by the Montgomery County TB and Heart Association and held at the National Institutes of Health; and the White House Conference on Children and Youth. HIC assisted a number of foreign visitors by arranging a series of meetings with appropriate NHI staff. These included a director of cardiovascular research from Japan, a Venezuelan professor of cardiology, and officials from Indian cardiological societies.

Programs were planned and arrangements made for three one-day Heart Seminars, conducted for a total of 40 professional persons from affiliates of the American Heart Association as an extension of a training period at the Association's headquarters in New York; for the one-day annual Seminar for Catholic University graduate students in cardiovascular nursing (also, a transcript from tape of the previous year's Seminar was edited for publication as part of the proceedings of the Cardiovascular Disease Nursing Work-

shop held by the University); for Heart Research Day, sponsored by the Montgomery County Heart Fund in cooperation with the National Heart Institute, a special project for 100 local physicians and community leaders; and for the visit of 40 high school science students to the laboratories and other parts of the Heart Institute as part of a program "Weekend With the Scientists" sponsored by the Heart Association of Maryland. HIC participated in the planning and development of a new program of summer fellowships in heart research for high school science students, to begin in 1961 under sponsorship of the National Heart Institute, the Montgomery County Heart Fund, Board of Education, and Health Department.

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NATIONAL HEART INSTITUTE

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Serial Number

2. BIOMETRICS RESEARCH
Branch

3. Harold F. Dorn
Branch Chief

During the calendar year 1960 the biometrics activity of the National Heart Institute was reorganized. The former Biometrics Research Section was replaced by the Biometrics Research Branch with an increase in the number of professional statisticians and expansion of the scope of its activities. The Biometrics Research Branch provides biometric consultation concerning the design, execution, and analysis of experiments and field studies, participates with other scientists in carrying out epidemiologic studies, and initiates and carries out research projects.

Consultation

Biometric assistance in the design of experiments and studies and in the analysis of data was given investigators not only in the Heart Institute but also in other parts of NIH. Harold Kahn and Dewey Shurtleff have had complete responsibility for the biometric aspects of the Heart Disease Epidemiology Study in Framingham, Massachusetts. Harold Kahn also has been responsible for designing the processing and analysis of the data collected by the New York City Medical Examiners' Study of Sudden Death and the Memphis Mortality Study conducted by the Epidemiology Section.

Jerome Cornfield has been a member of the Executive Committee for the Study of Diet and Heart Disease. This is a private group supported by a grant from the NHI and directed by Dr. I. H. Page of Cleveland. This committee is planning a controlled study to test the hypothesis that the substitution of polyunsaturated fats in human diets will significantly reduce the incidence of coronary heart disease.

Consultation to International Organizations

Jacob Lieberman spent six weeks from June 13 - July 23 in Geneva, Switzerland as a consultant to the Division of Health Statistics of the World Health Organization.

Harold Dorn was a member of a Study Group on Bilharziasis, convened by the World Health Organization in Geneva, July 16 - 25.

Conferences

Jerome Cornfield presented a paper on regression phenomena at the Symposium on Clinical Pharmacology sponsored by the Upjohn Company at Kalamazoo on October 10-11.

Harold Dorn and Jerome Cornfield participated in the Conference on Health Studies of Human Population held at the School of Public Health, Pittsburgh, Pa. on November 13 - 16 under the auspices of the National Advisory Health Council.

Harold Dorn presented a paper on the mortality of smokers at a Conference on Tobacco and Health held September 26 - 27 in New York City under the auspices of the New York Academy of Medicine and the New York State Academy of Preventive Medicine.

Interagency Committee on Medical Records

This committee, of which Harold Dorn is Chairman, acted on several matters referred to it by the Bureau of the Budget. Revisions were prepared of several clinical record forms used by Federal hospitals.

Participation in Activities of National and International Professional Organizations

Jerome Cornfield has been Associate Editor (for biostatistics) of the Journal of the American Statistical Association.

Harold Dorn participated in the activities of several National and international professional organizations as follows:

1. Advisory Committee on Statistics of the Pan American Health Organization.
2. Member, Board of Trustees, Population Reference Bureau.
3. Committee on Nomenclature, American Medical Association.
4. Research Advisory Council, American Cancer Society.
5. Committee on Epidemiology and Biometry, National Institutes of Health.
6. U.S. National Committee on Vital and Health Statistics.
7. Member, Board of Directors and Executive Committee of the Social Science Research Council.
8. Board of Directors, American Statistical Association.
9. Departmental Committee on Mathematics and Statistics, U.S. Dept. of Agriculture, Graduate School.
10. Committee on Classification and Staging, American College of Surgeons.

Harold Dorn continued his activities as General Secretary of the International Union Against Cancer. In this capacity he visited Moscow to discuss with the U.S.S.R. Organizing Committee plans for the Eighth International Cancer Congress which is scheduled to be held in Moscow during July 1962.

Revision of the International Statistical Classification

The International Statistical Classification of Diseases, Injuries, and Causes of Death promulgated by the World Health Organization is used by all member countries for the classification of causes of death published in official reports of vital statistics. The classification is revised every two years. Harold Dorn is Chairman of the committee responsible for preparing the suggestions from the United States for the next revision, scheduled to become effective in 1965. The section on cardiovascular diseases has long been considered unsatisfactory. During June 1960, representatives of the U.S. Committee met in New York with representatives of the corresponding Committee in Great Britain to draft a tentative revision of this section. This draft revision is being used in the two countries to code mortality and morbidity records in order to test its usefulness.

U.K. - U.S. Board on Cardio-Respiratory Diseases

Marked differences exist in official mortality statistics for coronary heart disease and chronic bronchitis in the United States and Great Britain. Chronic bronchitis, one of the common fatal diseases of men in Great Britain, is rarely reported as a cause of death in the United States. Coronary heart disease is more common in the United States, particularly among adult males between 40 and 54 years of age. Harold Dorn is a member of a joint U.S. - U.K. Board to investigate the reasons for these differences. The American members of this Board spent two weeks during March 1960 visiting British hospitals and medical schools in order to observe at first hand the problem of chronic bronchitis in Great Britain. Plans now are being made to organize comparable studies in the two countries in order to ascertain the extent to which the recorded differences in mortality rates reflect national differences in nomenclature and diagnostic methods or real differences in the incidence and nature of the two groups of diseases in the two countries.

NHI, Biometrics Research Branch (cont'd):

Publications

Larson, Carl L., Ribi, Edgar, Milner, Kelsey C., and Lieberman, Jacob, E., "A Method of Titrating Endotoxic Activity in the Skin of Rabbits", J. Exp. Med., III, 1-20, 1960

Baer, Paul N. and Lieberman, Jacob E., "Periodontal Disease in Six Strains of Inbred Mice," J. Dental Research, 39, 215-225, 1960.

Leone, Nicholas C., Lieberman, Jacob E. and Geiser, Patricia B., "The Effects of the Absorption of Floride, Gross Findings", A.M.A. Archives of Industrial Health, 21, 328-329, 1960.

Geever, Erving F., Leone, Nicholas C., Geiser, Patricia B. and Lieberman, Jacob E., "The Effects of the Absorption of Fluoride, Part I, An Investigation of the Pathology", A.M.A. Archives of Industrial Health, 21, 333, 1960.

Geever, Erving F., Leone, Nicholas C., Geiser Patricia B., and Lieberman, Jacob E., "The Effects of the Absorption of Fluoride, Part II, Microscopic Findings", A.M.A. Archives of Industrial Health, 21, 334, 1960.

Dekaban, Anatole S., and Lieberman, Jacob E., "Correlation of the Fetal Wastage and The Condition of the Offspring with Maternal State During Gestation and the Circumstances of Delivery in 4,156 Pregnancies." Part I. "Demographic Characteristics and Summarized Results of the Data." Part II. "Analysis of the Main Distributions of the Outcome of 4,156 Pregnancies."- In press - Am. J. of Obst. & Gyncology.

Feild, Lee E., Kreshover, Seymour J. and Lieberman, Jacob E., "Temporary Uterine Circulatory Arrest as a Cause of Abnormal Fetal Development" - In press - J. of Dental Research.

(Attachment 1)

Serial No. _____
1. Biometrics Research Branch
2.
3. Bethesda

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Part A.

Project Title: RELIABILITY OF DIAGNOSES OF CARDIOVASCULAR-RENAL DISEASES

Principal Investigator: Harold F. Dorn, Chief, Biometrics Research Branch

Other Investigators: I. M. Moriyama
Cooperating Units: National Office of Vital Statistics

Man Years (calendar year 1960):

Total: 1.0
Professional: 0.4
Other: 0.6

Project Description:

Objectives: To determine diagnostic methods used to establish the cause of death reported on death certificates whenever cardiovascular-renal diseases are involved. This is the first step in a project to measure the reliability and validity of diagnoses of cardiovascular-renal diseases recorded in official mortality statistics.

Methods Employed: A questionnaire requesting information concerning the medical history of persons certified as having a cardiovascular-renal disease at the time of death will be sent to the physician who certified the cause of death.

Part B included Yes _____ No x

NHI, Biometrics Research Branch, cont'd:

Major Findings: The questionnaire has been designed and tested on a sample of physicians. A revised questionnaire has been prepared and this will be sent to physicians during 1961.

Course of Project: The collection of information should be completed during 1961.

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Part A.

Project Title: RELATIONSHIP OF THE USE OF TOBACCO TO HEALTH

Principal Investigator: Harold F. Dorn, Chief, Biometrics Research Branch

Other Investigators: Katherine M. Herrold

Cooperating Units: Laboratory of Pathology, NCI

Man years (calendar year 1960):
Total: 6.8
Professional: 0.1
Other: 6.6

Project Description:

Objectives: To determine differences in mortality from specific causes of death among persons with varying habits with respect to the use of tobacco; to investigate factors other than tobacco-for example, occupation and residence- which may also account for the observed differences in mortality.

Methods Employed: Information concerning residence, occupation, and the use of tobacco is being collected from about 290,000 persons who held U.S. Government Life Insurance policies in December 1953. As each person included in the study terminates a policy, the Biometrics Research Branch is notified by the Veterans Administration. If the policy is terminated as a result of death, information is collected from physicians and hospitals concerning the causes of death and the methods of establishing these diagnoses.

Part B included Yes x No _____

NHI, Biometrics Research Branch, cont'd:

Major Findings: The death rate from all causes of persons who have used tobacco is 32 percent greater than that for persons who have never smoked. Persons who have regularly smoked only cigarettes have a death rate 58 percent greater than the rate for nomsmokers. The excess mortality for cigarette smokers is greater for heavy than for light smokers. The mortality rate for regular cigarette-only smokers is 10 times that for nomsmokers.

Course of Project: The collection and review of histological material for persons dying from lung cancer continued throughout the year. Collection of medical information concerning the cause of death of deceased policyholders is continuing.

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Part B.: Honors, Awards, and Publications

Publications

Dorn, Harold F., "Note on Cigarette Smoking and Lung Cancer", Public Health Reports 75, 582, 1960.

Dorn, Harold F., "Death Rates and Causes of Death of Smokers and Nonsmokers", in Press.

Zukel, William J., Dawber, Thomas R., Kannel, William B., Kahn, Harold A., and Dorn, Harold F., "Coronary Heart Disease and Mortality Among Smokers and Nonsmokers", in press.

(Attachment 1)

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1. Biometrics Research Branch
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Part A.

Project Title: STUDIES IN STATISTICAL INFERENCE

Principal Investigator: Jerome Cornfield, Biometrics Research Branch

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 0.15
Professional: 0.15
Other: 0.00

Project Description:

Objectives: (1) To develop a system of statistical inference that can be used in problems characterized by nuisance parameters, for which existing methods are inadequate; (2) to apply this system to particular problems in biostatistics of interest.

Methods employed: Mathematical

Course of Project: Three systems have been studied in detail. By the end of the year attention has become concentrated on Bayesian inference, with the a priori distribution determined by the condition that the a posteriori distribution lead to unbiased estimates. It has been proved that the unbiasedness condition uniquely determines an a priori function (for distributions continuous in the unknown parameters). It is planned to apply this system to reveal classical problems like the Behrens-Fisher problem.

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Part B.:

Publications

Roizman, Bernard, Hoggan, M. David, and Cornfield, Jerome, "Linear and Parabolic Estimates of the Titers of Herpes Simplex from Pock Counts on the Chorioallantoic Membrane of Embryonated Eggs", Virology, Volume 11, No. 3, July 1960

Smith, Willie W. and Cornfield, Jerome, "Utilizing Survival Time in Probit Analysis", Radiation Research, Volume 13, No. 4, Oct. 1960.

Cornfield, Jerome, Steinfeld, Jesse, and Greenhouse, Samuel W., "Models for the Interpretation of Experiments using Tracer Compounds", Biometrics, Vol. 16, No. 2, June 1960

Cornfield, Jerome and Haenszel, William, "Some Aspects of Retrospective Studies", J. of Chronic Diseases 11, 523-534, 1960

Serial No. NHI-
1. Geographic Pathology Section
2. Technical Services Branch
3. Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Methodology in Dietary Appraisal

Principal Investigator: Marjorie Grant Whiting

Other Investigators: Olive Hayes, Heart Disease Control, PHS, and Robert Harris, MIT

Cooperating Units: Heart Disease Control, PHS; Biometrics Research Branch, NHI; Computation and Data Processing Branch, DRS, NIH; Heart Disease Epidemiology Study, NHI; School of Public Health, University of Michigan; School of Public Health, University of Minnesota; and Department of Biochemistry, MIT

Man Years (calendar year 1960):
Total: 20.0
Professional: 5.0
Other: 15.0

Project Description:
Effort continues for improving methodology for collecting and processing dietary information for 1) epidemiological studies in large population groups and 2) investigations carried out in connection with anthropological studies of small, isolated, cultural groups.

1. For developing dietary appraisal methods suitable for epidemiological studies, the following can be reported:

   a. Field trials are being carried out at the University of Michigan for testing the survey instrument developed by them during their past year and a half of research. Their instrument is designed to obtain diet information pertinent to the study of cardiovascular disease which will be practical for use in large scale surveys.

b. In connection with the work of the Michigan group, a survey of literature has been compiled which describes and discusses published and unpublished reports of dietary appraisal methods. (contract # SA-43-ph-2434). A further evaluation of the literature is being prepared by the staff of this office in Bethesda.

2. For improving methods for handling and processing dietary data, the following are in process:

   a. Trials have been made of an electronic computer program for processing dietary data. Results from this analysis of dietary information will be used in studying the relationship of dietary characteristics to cardiovascular disease.

   b. Experimentation continues on the development of an inexpensive procedure (i.e., as cheap as man power), probably utilizing mechanical calculators rather than a digital computer, which will be suitable for the processing of large amounts of data from epidemiological studies. When completed, this procedure will be made available to research groups throughout the country.

   The staff working on a and b above include Olive Hayes, nutritionist, of Heart Disease Control Program and Robert Baird, statistician, Biometrics Research Branch, NHI. In the development of these two projects there has been close coordination with field epidemiological studies including Framingham, contractors from Heart Disease Control Program and grantees from the Grants and Training Branch, NHI. By utilizing facilities and staff available at NIH, we believe that we can develop techniques which will be used by many research teams.

3. Use of statistical methods in dietary appraisal studies. For further development of new approaches to the study of food intake and new ways of handling dietary data, a course dealing with this problem was included in the six-week summer session in bio-statistics at the University of Minnesota in the summer of 1960. In addition, a two-week course was held concurrently for persons unable to attend the full six weeks. J. Austin Heady from the London Hospital Research Laboratory, London, and Emil Jebe, University of Michigan, both of whom have had experience in the collection and analysis of dietary data, organized and taught the course. Class members included nutritionists and statisticians from federal, state, and local research studies as follows. For the two courses, 7 students came from USPHS, 7 from state health departments (Georgia, Louisiana, New York, and New Jersey), 4 from city or local health departments (Minnesota, Chicago, Palm Beach, and Evans County, Ga.), and 10 from special studies of cardiovascular disease.

   Consultants, during the development of the course and during the summer session, included members of the Sub-Committee on Dietary Appraisal of the Princeton Conference on Methodology of Cardiovascular Diseases held in April 1959. Financing was through contract # SA-43-ph-3749.

Plans are already in motion for the development of a similar course at a more advanced level for the bio-statistics summer session which will be in Minnesota again in the summer of 1961. This course will be financed through Research Grant #USPHS 2g-476. Dr. C. A. McMahon, Louisiana State University, will teach the course. He will be assisted by a staff member of the Biometrics Research Branch, NHI.

As in the previous year, in the organization of this course there is close coordination between the work of the Michigan group, our work on computer programs at NIH, and related research throughout the US and England.

4. Food Analysis (especially fatty acid content). For improvement of the nutrient values available for calculating food intake, especially the fatty acid content of ready-to-eat foods, a contract has been made with MIT for a research project for food analysis. Samples of foods characteristic of the American diet have been sent from a number of cooperating nutrition research projects. Gas chromatographic equipment for analysing fatty acid components of the fat content has been provided. A biochemist, Dr. A. K. Lough, Rowett Research Institute, Bucksburn, Aberdeen, Scotland, experienced in gas chromatography and interested in adapting existing methods for food analysis, will be at MIT for a year.

   We anticipate that, during 1961, suitable methods will be developed for analysing the fatty acid content of cooked foods and that a number of values will be obtained for commonly used foods which, through comparison with values in current use, will aid us in determining the direction of future work on this problem. This work is supported through contract #SA-43-ph-3737.

5. Preservation of food samples. Work is nearing completion on methods suitable for the preservation of field samples, collected in isolated communities, for shipment to laboratories in the US for nutrient analysis, particularly for fatty acid analysis. Problems involve the destruction of enzyme systems and of bacterial action without altering the composition of the fat present in the original sample. This project has been supported by contract #SA-43-ph-3731.

   Work will continue on this problem at MIT as a corollary to the problem described in item 4.

6. Collection of diet information from small, isolated, cultural groups.

   a. Development of method. In connection with studies of health and disease in isolated homogeneous social groups, attention has been given recently to the collection of diet information specific for individuals of the population.

      Through literature search and through consultation with anthropologists and a limited number of other professional workers who have attempted to obtain this type of quantitative data,

suggestions and directions are being written for standardization and improvement of methods suitable and practical for field workers.

If reliable data can be obtained which is representative of the diet of the group, a valuable tool will be available which can be utilized for further study of the relationship of diet to specific diseases.

b. Testing of method. There have been a limited number of opportunities for testing methods now in the process of development. An anthropologist, Jack Tobin, spent 2 months in Mexico locating and recording diet information for a selected cultural group. An anthropologist team, Dr. and Mrs. Gordon Gibson from the Smithsonian Institution, is now in Angola, Africa, working with isolated groups of a pastoral tribe, the Herero. Reports from both field projects will be available in 1961.

Part B included	Yes	No
	x	

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Trulson, Martha, et al
Report of Subcommittee on Methodology for Diet Appraisal, pp. 39-52 in Epidemiology of Cardiovascular Diseases Methodology (Report of Conference, Princeton, New Jersey, April 24-26, 1959) AJPH 50(10) (October) 1960.

Whiting, Marjorie Grant, and Leverton, Ruth M.
Reliability of Dietary Appraisal: Comparisons Between Laboratory Analysis and Calculation from Tables of Food Values. AJPH 50(6): 815-823 (June) 1960.

Becker, Beryl; Indik, Bernard P.; and Beeuwkes, Adelia M.
Technical Report, Dietary Intake Methodologies - A Review. (contract #SA-43-ph-2434) University of Michigan Research Institute Project 03188 (November) 1960.

Serial No. NHI-

1. Geographic Pathology Section
2. Technical Services Branch
3. Bethesda 14, Maryland

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Part A

Project Title: A Review of Russian Nutrition Literature During the Period 1950-1960.

Principal Investigator: Marjorie Grant Whiting

Other Investigators: Josef Brozek, Lehigh University, Bethlehem, Penna.

Cooperating Units: Russian Scientific Translation Program, DGMS, NIH; and Translating Section, NIH Library.

Man Years (calendar year 1960):
Total: 2.3
Professional: 0.3
Other: 2.0

Project Description:
To survey Russian nutrition literature during recent years.

Major Findings: Nutrition research as reported in Russian journals has been directed toward problems of food technology, nutrient analysis, and the use of scientifically planned diets for such special groups as athletes and workers in cold and hot environments. Considerable work is reported on the relationship of diet to chronic and other diseases.

Proposed Course: Reproduced copies of this compilation will be available on or about April 1, 1961. The contents included translations of nutrition symposia, a few selected full length articles, and abstracts from journals, mainly the Voprosy Pitaniia, (Journal of Nutrition) for the period 1950-1960.

Part B included	Yes	No
		x

ANNUAL REPORT OF FRAMINGHAM HEART DISEASE EPIDEMIOLOGY STUDY

Technical Services -- Epidemiology Section

The Framingham Heart Disease Epidemiology Study, National Heart Institute, Framingham, Massachusetts, is continuing its longitudinal prospective study of respondents, and is now beginning 12 years of observation of the Study group. The original Study objectives of identifying characteristics related to the development of coronary heart disease and hypertension are being pursued, and current hypotheses related to these diseases are being tested. The search for predictors of coronary heart disease is still the main objective. The susceptible individual is being identified with increasing clarity, and the strength of the various risk factors is being determined more precisely. In addition, information on the natural history of these diseases as they occur in the community is being accumulated.

Up to the present time, emphasis has been on collection of data, awaiting the accumulation of sufficient numbers of cases to allow detailed analysis of risk factors, time trends, and natural history. Sufficient numbers of cases have now been accumulated to enter into the phase of detailed analysis, and increasing publication of useful results is to be anticipated.

To this end, the statistical operation is being revamped to change the form of analysis to a life-table form more suitable to a longitudinal study. This will allow the inclusion of all persons developing risk characteristics, as well as more accurate handling of variable periods of follow-up. Survival data will also be more easily appraised. The Biostatistical Section is at present actively engaged in setting-up the new statistical model to be followed hence forth.

Examinations of the Study population for the sixth and seventh biennial examination continue at the usual rate of 200 per month.

Results of the six-year follow-up experience of risk factors related to the development of coronary heart disease have been submitted for publication and presentation to the American College of Physicians. This report deals with clinical characteristics, and is directed to the general medical profession rather than Public Health and Epidemiologically oriented groups. It is felt that the general medical profession should be made more aware of the preventive aspects of findings of cardiovascular epidemiology.

A preliminary analysis of eight-year follow-up data has been made and presented in October, 1960, to a meeting of the cardiovascular epidemiologists in Chicago. The findings in general have tended to strengthen previous information as well as providing new leads for more detailed analysis. When analyzed by the technique of life-table analysis, the eight-year data will be prepared for publication.

Visits to the Study project by investigators from many foreign countries have indicated that the Framingham Study is perhaps better known abroad than in this country. Many visitors have come seeking advice and counsel relative to

undertaking similar endeavors in their own countries.

A study of the relationship of bacteriuria to the development and presence of hypertension continues. The study is being done in co-operation with Dr. Edward Kass of the Boston City Hospital who is an acknowledged expert in this field. Urine specimens are being collected on all subjects participating in the Study. The relationship of other characteristics, such as obesity, family history of hypertension, marital status, salt intake, spouse relationships, among others, to the development of hypertension have been studied.

A combined study of the effect of smoking on morbidity and mortality from coronary heart disease has been designed. Data from Framingham and the Albany Study will be pooled. This has been made necessary by the lack of sufficient numbers to allow detailed analysis of this characteristic, as it relates to the development of coronary heart disease thus far in Framingham or Albany as independent studies.

An assessment of Dietary Methodology has been completed, and was presented at the New York State Health Institute in May, 1960. A method for the evaluation of long-term dietary habits of a population being subjected to prospective longitudinal study was presented. The reliability of the method was assessed, indicating that good agreement between two observers can be obtained. An estimate of the variability of dietary characteristics in the population has been obtained in a sub-sample so constructed as to maximize variability under existing hypotheses of the pathogenesis of coronary heart disease. It was concluded that important degrees of variability with respect to fat intake probably do not exist, owing to the lact of sufficient persons with "low" fat intake.

Dietary interviews on a subsample of 1000 persons has been completed. More detailed analysis and correlations with disease and other significant variables is underway. In addition, a study is being instituted to determine if a short form or dietary questionnaire can be developed, which would give information comparable to that obtained in the long form, with a view to using such a questionnaire on the entire Study population. Arrangement is also being made to do a detailed discriminant function analysis of all dietary characteristics obtained in the interview, to see if any combination of these relate to the presence of coronary heart disease and hypercholesterolemia, or to the subsequent development of these characteristics.

A study of the change in smoking habits in the population under study has been undertaken, to determine if any effect on smoking habits as a result of lay publicity of the relationship of smoking and lung cancer or heart disease can be demonstrated. This study is near completion.

Development of statistical methodology for handling longitudinal data has become an integral part of the Framingham Study. This has been necessitated by a lack of existing biostatistical methods to cope with many of the problems being encountered. Arrangements have been made to pursue discriminant function analysis and other more sophisticated and experimental statistical approaches, in co-operation with Dr. Lee Cady of the National Heart Institute and Dr. Max Woodbury at New York University using computers. This project has been

undertaken with the guidance and approval of Harold Dorn and Harold Kahn of the National Heart Institute Biostatistical Section. It is hoped that one of the contributions of the Framingham Study to the problems of epidemiologic studies in cardiovascular disease will be in the field of statistical methodology.

A study of the hormonal aspects of coronary heart disease epidemiology has been undertaken, with the co-operation of the Worcester Foundation. The sex differential in coronary heart disease has been one of the most striking findings in coronary heart disease. It is hoped that study of estrogen and androgen levels in subjects of the study will throw some light on the susceptibility to coronary heart disease amongst men. In conjunction with this, morphological and anthropological studies are being designed to assess the relationship of body habitus to hormone levels and to the development or presence of coronary heart disease. The latter is being undertaken with the guidance of Dr. Damon of the Harvard School of Public Health.

After many attempts, a procedure for the evaluation of emotional stress as a predisposing factor in the development of coronary heart disease has been devised. Self-administered interview forms exploring past history of traumatic experiences, personality factors, and anxiety manifestations have finally been completed, and are ready for pretesting.

Secular trends in coronary heart disease incidence, serum cholesterol levels, and hypertension have been studied, and are being prepared for publication.

Further attempts were made during the year to implement a comparative study of coronary heart disease and related factors in Hiroshima, (Atomic Bomb Casualty Commission), and Framingham. To this end, Dr. Finch, Dr. Darling, and Mr. Jablon of the Atomic Bomb Casualty Commission met with the Framingham Staff. It was decided that serum cholesterol, blood pressure, and ECG findings would be compared in the two populations. The original 1000 Japanese electrocardiograms have already been read at Framingham. It is hoped that the remainder of the Study project will be implemented in the near future.

Study of the relationship of rheumatoid arthritis to the development of coronary heart disease continues. This study is being carried out in cooperation with Robert Bent Brigham Hospital. Latex-fixation tests are being obtained on the entire population, as well as clinical and x-ray appraisal for evidence of rheumatoid arthritis. The latex-fixation tests will be repeated at least one time to see how long they remain positive, and to determine the incidence of new positives. In addition to the primary objective of demonstrating a possible relationship between rheumatoid arthritis and coronary heart disease, it is hoped that incidence and prevalence information for rheumatoid arthritis will be obtained, as well as information concerning the natural history of the disease.

During the year, the Framingham Heart Disease Epidemiology Study has moved to new and more adequate quarters in the Framingham Union Hospital. These quarters were built to house the Framingham Heart Disease Epidemiology Study at the suggestion of the Board of Trustees of the Hospital. The new quarters

will undoubtedly result in a more efficient operation of the Study project.

INVESTIGATORS:

Thomas R. Dawber, M.D., Medical Director
William B. Kannel, M.D., Senior Surgeon
Nicholas Revotskie, M.D., Surgeon
Miss Georgiana Pearson, Dietitian
Abraham Kagan, M.D.
Joseph S. Stokes, III, M.D. -- part-time only.

STATUS OF EXAMINATIONS, October 31, 1960

Number selected			6507
Number examined		(69%) --	4469
Plus SX			740
Total initial examinations			5209

Number Examined Each Round

Round Number	Total	Sample	SX
I	5,209	4,469	740
II	4,792	4,052	740
III	4,653	3,935	718
IV	4,541	3,843	698
V	4,416	3,745	671
VI	2,412	1,751	661
VII	166	104	62

Number Not Examined Each Round

Round Number II		417		417		0
Deceased	54		54			
Examined later round	196		196			
Lost	167		167			
Round Number III		556		534		22
Deceased	108		101		7	
Examined later round	184		174		10	
Lost	264		259		5	
Round Number IV		668		626		42
Deceased	168		147		21	
Examined Later round	132		120		12	
Lost	368		359		9	
Round Number V		793		724		69
Deceased	234		202		32	
Examined later round	67		53		14	
Lost	492		469		23	

FIELD EPIDEMIOLOGICAL RESEARCH SECTION

The aim of this section is to identify factors of man and his environment that relate to the occurrence of specific cardiovascular diseases. Studies begin with recognized disease and identify the population from which they arise.

Memphis, Epidemiology Laboratory. Studies are designed to identify differences in the kinds and amounts of cardiovascular diseases between white and non-white populations. Mortality studies, through analyses of death certificates of Memphis residents, seek information on time-place-person trends. Occupational, residential, and mobility patterns are being examined. The studies cover the 50 year period from 1911 through 1960. Coupled with these death certificate analyses, a pathological study of heart morphology contrasts whites and non-whites in a large autopsy population. Preliminary findings point to a greater degree of coronary occlusion among white subjects than among age comparable non-whites.

Rochester, Minnesota, Studies. In cooperation with physicians of the Mayo Clinic, a follow-up study of blood pressure levels and reactions to the cold-pressor test is under way. Rochester school children who were first examined in the 1930's comprise the group under study. These subjects, now 30-50 years of age, are being located and reexamined to measure the incidence of hypertensive disease and the potential value of the cold-pressor test as an indicator of subsequent disease.

New York City Studies. Family members of individuals who die suddenly and unexpectedly are being interviewed to identify symptoms and activities that precede death. Physical, emotional, and environmental factors that may lead to or precipitate death from coronary heart disease are being sought. Decedents from coronary disease are contrasted with subjects who died from other causes, in search of antecedent attributes of difference between these two groups.

Columbus, Georgia, Studies. In conjunction with the Tuberculosis Branch, PHS, studies are underway on obesity and heart size as they may relate to cause and time of death. Populations under study consist of cohorts of Columbus residents who were first examined in 1946 as part of a tuberculosis and syphilis survey in which photoflurograms and demographic data were collected. Study subjects are being classified as to obesity and heart size through photoflurogram measurements. Follow-up procedures utilize analyses of death certification.

Cincinnati, Ohio, Studies. A study of postpartum psychoses has been completed in which certain prenatal and perinatal factors associated with the development of these conditions were identified. The study contrasted patients developing psychoses within six months after childbirth, with otherwise comparable women that remained free of mental aberrations. The psychotic women were older than control subjects. They had shorter gestational periods and produced lighter infants. Larger proportions of psychotic women than controls were hypertensive during pregnancy or parturition, and larger proportions experienced dystocia. Presumably as a consequence of a less favorable gestational environment, the perinatal mortality rate was higher among infants of psychotic women. These findings suggest that the genesis of postpartum psychoses include a somatic disturbance.

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GRANTS AND TRAINING BRANCH

Research Grants Program:

In fiscal year 1961, an appropriation of $55,570,000 was made for research projects. As of December 1, 1960, 1,988 research grant applications have been recommended favorably by the National Advisory Heart Council in the amount of $38,917,725. After allowance is made for reserves already set up for special programs (primate and clinical research centers, institutional grants, international medical research, etc.), $718,275 remains for further grant awards following the recommendations of the National Advisory Heart Council at the March 2-4, 1961 meeting. At this final meeting, it is anticipated that more than 475 competing applications will be considered.

The objective of this program is to stimulate and support worthwhile research in cardiovascular and related areas and in so doing, to aid in the development of controls and ultimately, the means of prevention of heart disease in man. Since heart disease is a complex of many different diseases, the scientists must develop investigations in this area with a variety of approaches. Many studies supported by research grants are of a very fundamental nature, exploring the basic structure and function of the cardiovascular system. Other studies of a clinical level might deal with the application of the latest developments in cardiac surgery or drug therapy in a specific area of cardiovascular disease. All of the approaches must be pursued and supported if we are ever to realize the goal of total prevention of these maladies in man.

Even in the very recent past, a diagnosis of heart disease too often meant a sentence of a sedentary way of life, of part-time employment at best, and presumably of short life expectancy. Happily, that dismal foreboding is now largely dispelled; and more and more victims of heart disease look forward to complete recovery and a productive future. The basis for this optimistic outlook has been provided and is being fortified by findings of research workers throughout the world.

In managing the rather extensive cardiovascular research grant program, the Heart Institute utilizes the advice and guidance of experts in various fields of science to single out the meritorious projects for support. Also, following the advice of these outside experts, attempts are made to encourage research in fields which show particular promise or those which indicate a definite need for stimulation.

Heart Institute grantees attack research problems with precise weapons for detection, exploration, and conquest of disease. The clues are being conscientiously and thoroughly pursued, from the very basic questions of biochemistry, physics, and biology to the broader physiological and psychological

concepts involving animal experimentation and clinical studies. New ideas are continually cropping up, however, even as some old ones are being discarded, and the fertile field of investigation is urgently inviting new hands and minds for weeding out, for planting and cultivation, and for harvesting of the useful fruits of their labors. Some of the research accomplishments, described in the following pages indicate the avenues of investigation being used by the thousands of scientists, technicians, and auxiliary personnel whose work is supported by research grants.

Arteriosclerosis

Current medical research has implicated an overabundance of factors which alone or with accomplices may be the culprits in arteriosclerosis. The role of diet in particular has aroused considerable controversy, since various dietary schemes intended to reduce high blood lipid levels (linked intimately with atherosclerosis) have met with various degrees of success. Studies have suggested that the concept of a balanced diet should be revised to include consideration of the balance of saturated vs. unsaturated fats, of total fat intake to caloric intake, of fat to protein ratio, etc. It appears worthwhile to speculate the possible significance for public health and preventive medicine of this concept, and to pursue the promising leads that have been forthcoming from animal and human studies now in progress.

One of the most challenging problems facing research workers is the means for diagnosis of coronary disease before an actual coronary attack might occur. The concept of a "coronary profile" based upon blood cholesterol levels has been postulated and repeated measurements of such levels suggest that this index might have clinical value. Greater accuracy of diagnosis is being achieved through chemical determinations such as blood serum LDH (lactic dehydrogenase) which appears to be increased in every case of proved coronary attack. Improved procedures for visualization of the coronary vessels are eliminating many of the objectionable features of past techniques.

New drugs which will control lipid levels, or which will protect linings of blood vessels from arteriosclerotic substances are constantly being sought. The indictment of clotting and clot-dissolving disturbances points to the need for agents which will attack disorders of these mechanisms but which are subject to effective control by the physician. Long-term clinical trials will be needed to determine the extent of the usefulness of these drugs.

Because arteriosclerosis is not universally common, the factors which contribute to its prevalence among certain population groups and its non-existence in others are being sought. Studies of this kind can help to assign a proper place to the myriad of factors such as physical activity, obesity, emotional, economic and physical stress, as well as diet and heredity. Studies of environmental factors, too, such as climate, altitude and water hardness, are producing fascinating and significant correlations with the incidence of cardiovascular disease; and these avenues must be further explored.

Cerebrovascular Disease

Abnormalities in the cerebral blood vessels and in the vessels leading to the brain are a major cause of disability and death. The abnormal vessels occasionally rupture causing hemorrhage, but in most cases, their passageways are narrowed or occluded, thereby decreasing blood flow and causing damage to cerebral tissues. Studies are under way to allow early diagnosis of such conditions in the hope of averting such attacks. Visualization of the cerebral vessels by improved techniques will make it possible to select operable patients, with greater promise of survival. The hazards of thrombosis following small vessel surgery and the dangers of excessive anticoagulation are being firmly grappled with.

But--the nature of the vascular change which is limited to the vessels of the brain or affects these vessels more severely, the cause of hemorrhage, the relation of hypertension to the precipitation of cerebral attacks, the dynamics of intracranial vascular conditions, basic knowledge of the metabolism of brain tissue--these are comparatively unexplored areas of investigation with great potential for medical progress.

Hypertension

Even the definition of what constitutes essential hypertension remains a formidable question. Arteriosclerosis, cardiac enlargement, renal changes and other structural alterations which may develop in hypertension can and do occur in the absence of hypertensive disease. The kidneys, the central nervous system, heredity, enzymatic and hormonal factors are believed to participate in the pathogenesis of the disease, but the initiating or precipitating factor is not clear. Evidence suggests that there may be multiple abnormalities present, and that while any one abnormality may not be of sufficient magnitude to produce hypertension in itself, combinations however may interact and become causative.

Drugs which are capable of lowering blood pressure and providing relief of troublesome symptoms represent significant contributions to the problem, but essentially treat the manifestation of the disease rather than the disease itself. What is more, many antihypertensive drugs have proved to be of limited usefulness because of "side effects" and must be subjected to more careful scrutiny. A three year study is beginning in five Public Health Service hospitals to test the effectiveness of various drugs in the treatment of essential hypertension on large numbers of outpatients.

Words that assume an association between life stress and heart disease have become rooted in everyday language. The mechanics involved in cerebral "stroke" are somehow related to "blowing your top". The business executive who suffers a heart attack is presumed by many to have been under extreme tension prior to it. But more and better evidence is needed before psychologic stress is established as either a remote or proximate cause of hypertension or other cardiovascular disease. Studies have indicated that associations do exist, but whether transient elevations in cholesterol, for example, lead to the development of atheromatous lesions is still a mystery. The critical experiments remain to be done.

Rheumatic Fever and Rheumatic Heart Disease

Following observations that streptococcal infections are intimately associated with the occurrence of acute rheumatic fever, a mass of clinical, epidemiological and immunological data supporting this concept has accumulated. Further studies are needed to explain the correlations with age, with geographical and seasonal variations, and with family incidence. The role played by the streptococcus in the production of rheumatic fever remains obscure. There is reason to think that toxicity may account for injury to tissues resulting in the manifestations of rheumatic fever; many feel that rheumatic fever may have an allergic basis; and it has been suggested that a virus infection may accompany the streptococci as they invade the tissues. All of these concepts and others are subjects for continuing investigation. A prophylactic attack on the disease, in the form of early diagnosis, and antibiotic therapy are stop-gaps.

The prevention and treatment of the devastating damage to the heart valves in many patients as a result of rheumatic fever poses many of the most challenging problems in medical research. Recent studies indicate that patients initially free from cardiac involvement are not likely to develop rheumatic heart damage during subsequent attacks. This suggests that the rheumatic host has a specific and persistent susceptibility toward or against involvement of the heart valves. This finding may enable a reduction in the duration of treatment for valvulitis-free patients, if these conclusions are borne out by further studies.

Congenital Heart Disease

Before the advent of circulation of blood outside of the body for cardiovascular surgery, children born with defects within the heart were viewed with little hope for survival beyond their teens. The possibility of direct vision repair of lesions such as a damaged septum has completely changed the patients' prognosis. Constant improvement in equipment and techniques is resulting in a progressive lessening of operable risks. The successful use of hypothermia represents a tremendous stride, but difficulties remain to be overcome. There remain many forms of the disease where surgical procedures have not been successful.

Much effort is being directed toward a clarification of the causes of these inborn malformations. The production of congenital defects in animals by experimental dietary manipulation and other means has provided the means for malformation research which was previously impossible. The use of genetically inbred animals is helping to ascertain the role of heredity. The influence of fetal conditions, and of environmental factors such as radiation are being scrutinized, and the results are expected to illuminate some of the questions relative to the human disease.

The early diagnosis of congenital defects continues to present a challenge. It has been pointed out that the electrocardiogram may appear essentially normal despite critical abnormalities. The use of radioisotopes

and radiographic blood vessel visualization techniques are making detection possible, but the wrinkles in the use of these techniques must still be ironed out.

Heart Failure

Failure occurs when the heart fails to meet the demands of the body. In the more common instances of failure this difficulty with the pumping action is chronic, and the heart fails to meet only peak demands. In some situations, such as in severe anemia and hyperthyroidism, the demands may be sustained at a high level, and although the heart may respond, inadequacy and failure may occur. In other instances functional loss of a large area of heart muscle as a result of coronary occlusion, or an overload caused by severe hypertension may induce failure. The heart fails because the muscle is not functioning correctly, but the nature of the failure of the cardiac muscle is still open to considerable controversy. Studies of the metabolism of cardiac muscle are beginning to clarify the chemical processes involved.

The faulty excretion of fluid and the respiratory symptoms common to patients with failure are being attacked effectively, but these are only symptomatic treatments. Corrective surgery for cardiovascular lesions contributing to heart failure is becoming more realistic with the development of specialized techniques for diagnosis such as cardiac catheterization and radiographic blood vessel visualization.

Cardiovascular Aspects of Aging

Workers in the field of aging are facing a double challenge -- that of adding years to life and of adding life to years. It is recognized that many chemical and physical variations occur during aging, but whether these variations are the causes or the consequences of senility is still in doubt. Most of the diseases of old age are subject to experimental approach, and the use of such methods as retarding growth in animals has opened up new areas of attack. Species differences play an important part in age changes and comparative pathological studies are needed. Nutritional studies as well as studies of the effects of genetics, exercise and environment are in progress. Basic studies of elastic tissue, and measurements of hormones and enzymes in young and old are revealing significant differences which are stimulating further scrutiny.

The management of the older adult, with a view to improving the quality of his life, is an ubiquitous charge. Psychologic, sociologic and biologic considerations complicate the problem, and indicate the need for a multifaceted approach.

Blood Diseases: Coagulation Defects

Clotting of blood can be life-saving in one instance and devastating in another. Alterations in the clotting mechanisms have been blamed in large part for many of the critical cardiovascular impairments, including cerebrovascular accidents, coronary thrombosis and thrombophlebitis. The components

of the blood, as well as the other substances for which blood is a vehicle, are being systematically manipulated to unravel these phenomena. Confusion among investigators and physicians in terminology applied to the many blood clotting factors is being decreased by international committees on nomenclature which will recommend uniform terms.

Previous work with anticoagulants has been designed principally to restrict extension of existing blood clots or to diminish the incidence of new clots. More attention is now being directed to clot-dissolving agents which can effect the rapid resolution of acutely formed clots. Enzyme therapy in the form of plasmin or streptokinase have added a major tool in the therapeutic management of acute thrombophlebitis and pulmonary embolism, and is showing promise in the therapy of myocardial infarction. Additional study is needed to help clarify the reasons for a satisfactory response to fixed dosages in some individuals, and no response in others.

Standardization of agents and the eradication of undesirable side effects are pressing needs. Clinical trial alone is not adequate as a test system, and methods must be devised to determine the optimal biochemical conditions for effective clot dissolution.

Cardio-pulmonary Disease

The lungs, by virtue of their unique position in the circulatory system are a sensitive indicator of inadequate performance of the heart. More adequate methods for appraising pulmonary function, such as cardiac catheterization, have made it easier to distinguish between primary disease of the lungs and congestion secondary to disease of the heart. Breath-holding tests are used as an indication of cardiac status and may be used to differentiate functional from organic cardiovascular disease.

Investigations into many aspects of cardio-pulmonary physiology and pathology are underway. Research probing the causes and prevention of pulmonary complications following anesthesia and surgery; the natural history of emphysema, many aspects of which are still unknown; and the causes of newborn death associated with pulmonary and cardiac dysfunction are among many studies which will help to bring the picture of cardio-pulmonary relationships into sharper focus.

Other Areas

In addition to the disease categories above, active investigation is being supported and expanded in a number of other areas important to the overall field of cardiovascular medicine. Investigations continue at a basic clinical level in surgery to ultimately attain improvements in operative procedures which can be utilized for a variety of cardiovascular malfunctions. Geographic pathology, a long-term research area, is contributing much to the elaboration of the role of geographic, ethnic, and nutritional factors on the incidence of heart disease. The grants program will encourage and assist research whenever feasible in such wide fields as physiology, pharmacology, biophysics, biochemistry, hematology, pathology and genetics. Information

from studies of these fundamental types helps form a basis for biological generalizations useful in a broad attack on cardiovascular disease problems.

Primate Research Centers

Interest in primate research centers has been engendered by two facts: (1) the need in cardiovascular research to employ as experimental subjects species that are structurally and physiologically similar to man, and (2) the non-existence in the United States of any facilities of truly national character for research on subhuman primates.

Last year the Congress considered the need for establishing national primate research facilities and authorized two million dollars for this purpose. This enabled the first grant award for the establishment of a regional primate center. When construction is completed, this center will provide an excellent environment for research designed to advance all possible areas of knowledge on the biological characteristics of the rhesus monkey and other appropriate species throughout the lifespan of the primate.

Experience gained in working out the details of the establishment of the first primate research center is enabling the more efficient handling of requests for additional centers. With the $7,000,000 made available in FY 1961, the Heart Institute plans to support the one center started last year and to proceed with orderly establishment of possibly three additional centers of about the same magnitude as the prototype.

Clinical Research Centers

A cardiovascular clinical research center including both a broad research program and supportive services, will provide a stable organizational framework in which investigators from all disciplines necessary for a comprehensive attack on cardiovascular disease problems can enter into most effective collaboration, with all necessary diagnostic and research equipment resources immediately available. Such a center will permit the most comprehensive and effective research effort possible, including opportunities for cross-fertilization.

These centers will be characterized by diversity and flexability. The facilities supported by a center grant, providing for the complete and carefully controlled clinical care of patients, will permit both acute and long-term studies by investigators with a variety of research interests.

Planning for use of the $6,000,000 allowed for centers in FY 1961 revolves around a fairly sizable group of formal requests already on hand and soon to be reviewed by the necessary advisory groups. Programming also includes continued support of the general research center programs initiated last year by the Division of General Medical Sciences.

Training Grant Program:

An appropriation of $11,970,000 was made in Fiscal Year 1961 for research training grants at the undergraduate and graduate levels. As of December 1, 1959, 321 training grants have been awarded in the amount of $9,532,239. It is probable that approximately 48 training grant applications will be considered at the March 1961 meeting of the National Advisory Heart Council. Of the total appropriation, $2,366,000 was allowed specifically for the completion of the adjustment of starting dates on all graduate training grants. This procedure was started on a small scale last year with the plan designed to give program directors at the schools, more flexibility in arranging curricula, recruiting faculty and bringing in trainees.

The training program of the National Heart Institute has the dual objectives of (1) improving the quality of both basic and clinical research training in fields concerned with the study, prevention, and treatment of cardiovascular disease, and (2) attracting promising young students to careers in medical research in order to increase the national supply of cardiovascular scientists, teachers, and highly trained physicians. Training programs are supported at the medical undergraduate level, the graduate level and at various levels where training is important to the production of future medical research scientists in the cardiovascular field.

Undergraduate Training Program

The purposes of the undergraduate training grant is to support the medical schools in providing better teaching of the subjects related to the cause, recognition and management of cardiovascular disease. With this additional support the schools are able to provide better teachers who can devote more time to teaching, and better materials with which to teach. Experience has proven that schools with this support are able to do a much better job of teaching. As a direct consequence, the physician--graduate of today--has received a much more thorough training in the fundamental basis of disease as well as better methods of diagnosis and treatment, than earlier graduates. This program has not only raised the standard of competence of the profession as a whole, it has also attracted more physicians into seeking more advanced training and experience. The undergraduate training program has also stimulated and strengthened cardiovascular research in the schools and has enabled them to provide more comprehensive and intensive training in the clinical and public health aspects of cardiovascular disease. It has interested many medical students in medical research by making it possible for them to participate in the research activities of their schools. These beneficial effects of the undergraduate training grants will continue to be in force in the schools.

Graduate Training

Graduate training grants support special training programs in basic and clinical cardiovascular research areas in medical schools, universities, and other training institutions. The grants, handled locally by the program directors, cover certain costs of training and research and pay stipends to qualified candidates selected by the institution. They help to provide greater stability to meritorious training programs in various basic and

clinical disciplines in the cardiovascular field, and serve as a stimulus to research interests and activities of young medical scientists in the university and medical school environment. This type of grant is an effective means of helping to produce research personnel in areas of manpower shortage. In addition to reducing the primary manpower shortage, these programs aid in bringing the basic and clinical scientists together in broader study of cardiovascular diseases through training involving multiple disciplines and several departments. Training programs are utilized to establish instruction in new or undeveloped disciplines to take advantage of unusual opportunities and new approaches.

In order to provide training in the basic scientific disciplines, which are so vital to the successful prosecution of cardiovascular research, programs have been established in various preclinical departments (biochemistry, biophysics, and physiology). In such programs, a Ph.D. may receive specialized training in the cardiovascular aspects of his discipline, or a clinician may receive fundamental training which will strengthen his clinical research activities. Some of these programs are a combined effort of several preclinical departments, aimed at producing research workers with a trans-disciplinary approach to cardiovascular problems.

Cardiovascular lipid research is a good example of a preclinical area being given special attention by the Heart Institute because of obvious manpower shortages. This field includes three major areas in which there is a special need for more trained researchers: (1) newer methods of lipid analysis such as gas chromatographic and radioisotope techniques; (2) biochemical and nutritional aspects of atherosclerosis, particularly as related to lipid metabolism; (3) investigative studies on the relationship of hormones to lipid metabolism.

The National Heart Institute supports programs which provide specialized research training for the physician who has completed his formal medical training and wishes to pursue a career in cardiovascular research and teaching. These programs provide training in the application of experimental diagnostic and therapeutic techniques to clinical investigation. Many of these programs have used the multi-departmental approach to training much the same as in the preclinical programs described above. By combining the training resources of several clinical departments, it enables the various institutions to provide well-structured, diversified training programs from which competent clinical investigators will emerge.

High on the priority list of clinical research training areas, is the field of drug evaluation or clinical therapeutics. Because of the large number of new drugs resulting from research efforts in the cardiovascular field, there is an urgent need to train physicians who are competent to assess a new drug's effectiveness and potential harmful effects. It is an established fact that the medical schools do not have the personnel qualified to test and evaluate these agents adequately or to teach the methods to others. Accordingly, the Heart Institute has established and supports programs which give solid training in pharmacology and experimental design in order to increase the number of people qualified to conduct clinical therapeutic trials and offer instruction in this vital area.

In the cardiopulmonary area, the Heart Institute is supporting training programs which have incorporated basic research techniques through the cooperative efforts of such disciplines as pathology and physiology in the study of clinical cardiovascular pulmonary problems. Many of these programs involve advanced training in mathematics, physics, physical chemistry and statistics, as well as training in modern techniques of clinical cardiovascular evaluation.

During recent years there has been a weakness of research activity in pediatrics. This has been reflected by a lack of new knowledge concerning problems of cardiovascular disease in children -- notably congenital cardiac defects. To stimulate research in this area, the Heart Institute is developing qualified investigators through the support of training programs in pediatric cardiology. These programs offer training in diagnostic methodology (catheterization, dye dilution methods, radioisotopes, etc.) to equip the trainee with the necessary skills for conducting research in the fundamentals of cardiovascular problems prevalent in children.

Some of the other clinical research areas displaying shortages of well trained investigators, and to which the Heart Institute is giving special attention, are: experimental cardiovascular radiology; hematology; surgery; and cardiorenal research.

Fellowship Program:

An appropriation of $3,100,000 was made in Fiscal Year 1961 for postdoctoral, special, and senior fellowships and career research professorships. As of December 31, 1960 a total of 202 fellowships in the amount of $1,490,023 have been awarded. It is probable that about 300 competing applications will be considered for award during the remainder of the fiscal year.

The objective of the research fellowship program is to increase the number of trained heart researchers, and to assure the continuing flow of skilled and imaginative workers into the cardiovascular field. Only the most promising young scientists are selected for assistance in obtaining fundamental and advanced scientific training and supervised research experience. Candidates are carefully screened by the sponsoring institution and by selection panels within the National Institutes of Health and the Heart Institute. Attempts are made: to encourage research interests early in the postdoctoral training careers of young persons giving promise of becoming competent research scientists; to enable more mature investigators to obtain additional or specialized research experience, further developing their own research skills; and to provide stabilizing support for the advanced investigator in an attempt to ensure retention of the most qualified individuals within the field of cardiovascular research.

Since the Division of General Medical Sciences has assumed the responsibility for support of all predoctoral and part-time medical student fellowship awards, the Heart Institute, during the current year, is concentrating its program on the postdoctoral and more advanced investigator. In both the basic and the clinical disciplines postdoctoral fellowships are helping to train

future research scientists. This program is encouraging young physicians to obtain research training and experience that will enable them to utilize their medical knowledge more effectively in research on cardiovascular disease and is facilitating the training of basic research in disciplines important to the field.

Through the special research fellowship award, the more advanced scientist is enabled to attain improved methods and skills by means of unusual training experience in research laboratories other than his own. The more flexible criteria and wider stipend range available here presents a unique opportunity for making selective contributions to the research manpower pool. Special needs in the heart field, for example, are being met by bringing into a neglected research area the competent research scientist, at the advanced or senior level, whose skills are needed to facilitate development of the area. The special fellowship has proven to be very adaptable in the sabbatical leave situation, enabling the advanced scientist to make the fullest possible use of this time away from his own institution.

In addition to permitting some expansion of both the postdoctoral and special fellowship programs, increased funds this year have enabled the establishment of two significant new programs. These are the senior fellowship and the career research professorship awards. Both are designed to aid in stabilizing research careers for the more advanced and highly competent investigator in the field of cardiovascular disease. The establishment of the career professorship award is a large step in the direction of alleviating the shortage of full professorial positions in many medical schools due to lack of funds. Through this type of award, the medical schools will be able to recruit persons of exceptional professional calibre displaying promise for productive careers in cardiovascular research and teaching.

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1960

1. DIVISION: Special Health Services

5. DIVISION PROJECT NUMBER SHS-HD-9

2. BRANCH: Heart Disease Control Program

DIVISION CONTRACT NUMBER SAph 73812

3. SECTION: Rheumatic and Congenital Heart Diseases

4. LOCATION OF PROJECT: Chicago, Illinois

6. DATE WHEN INITIATED April 1955

7. PROJECT TITLE: Study of heart sound recorder in detection of heart disease

DATE WHEN REVISED October 1959

8. INVESTIGATORS: Dr. Carl Marienfeld
Dr. Arthur Rikli
Mr. Philip Enterline
Dr. Martin Levy

9. PARTICIPATING ORGANIZATIONS: Chicago Heart Association, Chicago Board of Health, Chicago Board of Education, Chicago Medical Society

10. OBJECTIVE AND RESEARCH PLAN: The Project is a research study designed to develop, experiment with, and field test a technique to screen large numbers of children, at a rapid rate, for possible cardiac abnormalities, manifested by acoustical phenomena.

This demonstration and testing study was begun on April 20, 1959 and at present approximately 33,000 Chicago elementary school children--from kindergarten through 8th grade--have been tape-recorded and read. Follow-up of nonrespondents, tabulation of data, and statistical analysis are presently being completed.

VOLUNTARY PROTECTIVE AGENCIES

1. DIRECTION: Special Medical Services
   Date: August 3, 1960

2. MEANING: Heart Disease Control Program
   DIVISION CONTRACT NUMBER
   SAG-HD-9

3. DUTIES: Monumental and Congenital Heart Diseases
   Zip 71312

4. LOCATION OF PROJECT: Chicago, Illinois
   6. DATE WORK STARTED
   April 1960

5. PROJECT TITLE: Study of heart sound recorded in auscultation of heart disease
   DATE WORK REVIEWED
   October 1959

7. APPLICATIONS: 9c. Cert Cardiologist
   9c. Arrhythmias
   9c. Mitral Stenosis
   9c. Normal Duty

8. AUTHORIZING ORGANIZATIONS: Chicago Heart Association, Chicago Board
   of Health, Chicago Board of Education,
   Chicago Medical Society

9. OBJECTIVE AND RESEARCH PLAN: The Project is a research study designed
   to develop, improve with, methods and a technique to screen large
   numbers of children, at a small cost, for possible cardiac abnormality,
   manifested by auscultation/phonocardiography.

   This demonstration and testing study was begun on April 20, 1959 and at
   present approximately 37,000 children (largely school children--from
   kindergarten through 8th grade--have been tested and read. Follow-
   up of questionable cases, individual data, and statistical analysis are
   presently being completed.

PROJECT CONTROL RECORD

SECTION I

PROJECT NO. HD-10

CONTRACT NO. SHS-HD-10

1. TITLE Nutrition Study of Seventh-day Adventists

2. TYPE Research

3. SECTION Atherosclerosis

4. RESPONSIBLE PERSONS (HDCP) 1. Marjorie Cantoni, 2. Olive Hayes

City	State
5. LOCATION Washington	D. C.

6. RESPONSIBLE PERSON (Field) Washington Sanatarium and Hospital

7. ADDRESS (Mailing) Takoma Park, Md.

8. COOPERATING AGENCIES: Washington Sanatarium and Hospital

9. DATE	(a) Contract Approved:	(b) Project Started: 11/55	(c) Project Completed:

SECTION II

1. BACKGROUND: Objective: To compare the degree of coronary atherosclerosis in vegetarians and non-vegetarians of the same age, sex, and race.

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-14

5. PROJECT TITLE
Trends in prevalence of rheumatic heart disease among college students.

6. INVESTIGATORS
Dr. Roy P. Sandidge, Jr.
Miss Margaret Evans

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
January 1956

9. OBJECTIVES
To determine trends in prevalence of rheumatic heart disease as detected in entering freshman college students over a period of five years. Further, to determine the extent of use of prophylactic measures to prevent recurrences of rheumatic fever among this group.

10. PRINCIPAL RESULTS
During the first two years of the proposed five-year study, data were reported on 176,588 entering college freshmen. Analysis of the data showed that there were 2,866 students who had a definite history of rheumatic fever and/or definite rheumatic heart disease, for a rate of 16.2 per 1,000 students examined. Only 288 of these students were following a regular program of anti-streptococcal prophylaxis at the time the examination was made.

11. PUBLICATIONS
A paper reporting the results of the first two years of the study was presented at the annual meeting of the American College Health Association in May 1959 and will appear in a subsequent edition of the Association's journal, Student Medicine. A second paper is now being written to be offered for publication in the American Journal of Pediatrics.

PROJECT CONTROL RECORD

PROJECT NO. SHS-HD-16

SECTION I CONTRACT NO.

1. TITLE North Dakota Coronary Disease Study

2. TYPE Research

3. SECTION Atherosclerosis

4. RESPONSIBLE PERSONS (HDCP) Philip Enterline and Jean Pekover

5. LOCATION Grand Forks, North Dakota

6. RESPONSIBLE PERSON (Field)

7. ADDRESS (Mailing)

8. COOPERATING AGENCIES: North Dakota State Health Dept., North Dakota Heart Asso., Grand Forks and Devil's Lake Medical Societies

9. DATE (a) Contract Approved: (b) Project Started: Sept. '56 (c) Project Completed:

SECTION II

1. BACKGROUND: A longitudinal study of cohorts of males 35 and over from the general population to obtain information on:

   1. Incidence of newly manifest clinical coronary disease defined as angina pectoris, coronary insufficiency, and myocardial infarction occurring within a six-county area, population 106,000, of North Dakota.
   2. Compare farming and non-farming population for incidence of coronary disease.
   3. Correlate occupation, physical exercise, smoking habits, diets and other factors.
   4. Obtain long-term prognosis of new coronary disease by yearly follow-up and by checking death certificates.
   5. To relate subsequent morbidity and mortality from coronary disease to certain base-line environmental characteristics established by the census.

2. ESTIMATED DURATION: indefinite

3. SUPPORTING AGENCIES:

   a. HDCP (Contract) Amount: $11,050.00

PROJECT CONTROL RECORD

PROJECT NO. SHS-HD-17

SECTION I

CONTRACT NO.

1. TITLE Relationship between Obesity in Childhood and Obesity in Adult Life
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSONS (HDCP) Dr. R. C. Arnold and Marie Nordsieck
5. LOCATION Hagerstown, Md.
6. RESPONSIBLE PERSON (Field) Washington County Health Department
7. ADDRESS (Mailing) Hagerstown, Md.
8. COOPERATING AGENCIES: Washington County Health Department
   PHS-Division of Public Health Methods
9. DATE (a) Contract Approved: (b) Project Started: 8/57 (c) Project Completed: 2/60

SECTION II

1. BACKGROUND: Hagerstown, Maryland has been used by the Division of Public Health Methods as an experimental field office for the purpose of testing methods and techniques. Among the large accumulation of records in the repository, records of weight and body measurement of school children in Hagerstown 20 years ago were found to be available and complete enough for study.
   The objective of the study was to determine if obese children tend to become obese adults, and to determine if in a group of obese adults, weight status as children is related to weight reduction problems in adults.

2. METHODOLOGY: Follow-up of this cohort was quite productive and a large number of the individuals originally examined as school children 20 years ago were available for re-examination and study. It was found that overweight children tend to become overweight adults more often than children of average weight. The difficulty that obese adults experience in weight control may be noted in the fact that overweight children tend to become overweight adults. It seems reasonable to suggest that part of the failure of traditional weight control activities is due to over-emphasis on adult eating habits.

3. ESTIMATED DURATION: Completed 2/60 (Paper published - Public Health Reports (March 1960 - Sidney Abraham, Marie Nordsieck
4. SUPPORTING AGENCIES:

   a. HDCP (Contract) Amount: $1,000.00

   b. OTHER AGENCIES:

1. [illegible]

2. [illegible]

3. [illegible]

4. [illegible]

5. [illegible]

6. [illegible]

7. [illegible]

8. [illegible]

9. [illegible]

10. [illegible]

11. [illegible]

12. [illegible]

13. [illegible]

14. [illegible]

15. [illegible]

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-18

5. PROJECT TITLE
Overweight vs. Obesity as related to cardiovascular disease.

6. INVESTIGATORS
Dr. H. A. Tyroler
Mr. Sidney Abraham

7. LOCATION OF PROJECT
Asheville, North Carolina

8. DATE PROJECT INITIATED
May 28, 1957

9. OBJECTIVES
To evaluate the significance of body fat content as distinguished from overweight per se as a factor related to the development of cardiovascular disease, and as a factor related to serum cholesterol levels and blood pressure levels.

10. PRINCIPAL RESULTS
Total serum cholesterol levels of the study group are in agreement with the levels found in high serum cholesterol populations. Too few cases of cardiovascular disease have developed thus far to warrant extensive analysis.

11. PUBLICATIONS
None

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-19

5. PROJECT TITLE
Cardiovascular Mortality by Geographic and Related Factors, particularly by Country and State of Birth.

6. INVESTIGATORS
Mr. Philip E. Enterline
Mr. Herbert I. Sauer

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
July 1957

9. OBJECTIVES
To compare cardiovascular mortality rates of (a) native whites and (b) foreign-born whites (total and by specific country of birth):
(1) for different sections of the United States;
(2) with countries from which most of the migrants to the United States have come;
(3) by State of birth of the native white
for the purpose of determining more precisely the differences in these rates, and to provide a basic framework from which to continue the search for factors responsible for these contrasts.

10. PRINCIPAL RESULTS
For foreign-born males in the Middle Atlantic States, 1950, the Italian-born have the lowest death rates for coronary heart disease, all cardiovascular diseases, and also for all causes. Those born in Ireland have the highest rates for all cardiovascular diseases and also for all causes.

Mortality rates by metropolitan areas and economic subregions present with more precision a pattern similar to that of death rates by State. Most of the metropolitan areas with high coronary death rates are near the ocean. The economic subregions, consisting of rural areas and small cities, show wide variation, with the lowest rates chiefly in the Great Plains area and with the highest rates generally near the coast. In general, the metropolitan areas have much higher rates than do the non-metropolitan areas.

Coronary heart disease death rates by State of birth generally follow patterns similar to those by State of residence. Those who

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Quarterly Jupiter Afternoon Cloudy Reason By Street We Black Woodwind Chosen Why

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Dear Rise Hear. To Crystal Rise Multiply Sugar Bulletin Sugar Quarterly Dear Tomorrow Rise Hear. To Crystal Rise Multiply Sugar Bulletin Sugar Quarterly
Crystal Rise Hear Fishing Dear Cry Mud Wish Rise Bulletin Sugar Quarterly Rise
Sugar Dear Acid Crystal Rock Cry Mud Wish Rise Bulletin Sugar Quarterly Rise Tomorrow Rise Hear. The Accountant Bulletin Conducting Mud Rise Tomorrow Rise Hear. The Accountant Bulletin Conducting Mud Rise
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Accountant Rise Hear. Mouse Of Fine Differentiate Strong Rise Engineer Sugar Quarterly Rise Accountant Rise Hear. Mouse Of Fine Differentiate Strong Rise Engineer Sugar Quarterly Rise

Tomorrow Rise Hear. Mouse Of Fine Differentiate Strong Rise Engineer Sugar Quarterly Rise Accountant Rise Hear. Mouse Of Fine Differentiate Strong Rise Engineer Sugar Quarterly Rise

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(3) Bridge Tomorrow

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Tide Tide Increase Go Tide Multiply Cook Increase Ear Quarter Tide Multiply Cook Decrease

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And (3) Purchase Modern Ear Tide Go Tide Multiply Cook Increase Ear Quarter Tide Multiply Cook Decrease
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8. OBTAIN:

Washington, D. C.

7. LOCATION OF RECORDS:

Dr. Herbert T. Ohmit
Mr. Philip E. Rutledge, Jr.
Mr. Robert L. Ohmit

6. INVENTORY:

Electronically Connect Study Ear Quarter Tide Multiply Cook Decrease
Quarterly Accurate Accept(s) Of Coordinate And Signal Increase.

5. PHYSICAL UTILITY

Under Disease Chemical

(59-60-1)

4. PROCESS:

Square Quality Jupiter Tomorrow

3. [illegible]

2. [illegible]

10. PRINCIPAL RESULTS (Cont'd) SHS-HD-19

were born in New York who moved away have death rates almost as high as those who remained in New York. Likewise, those who moved away from North Dakota have rates almost as low as those who remained in North Dakota.

11. PUBLICATIONS

A paper, "Are Geographic Variations in Death Rates for the Cardiovascular Diseases Real?" has been accepted by the Journal of Chronic Disease. (Sauer and Enterline)

A paper, "Death Rates for Coronary Heart Disease in Metropolitan and Nonmetropolitan areas of the United States, 1949-51," has been submitted to Public Health Reports. (Enterline, Rikli, Sauer, and Hyman)

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-20

5. PROJECT TITLE
Twenty-year Follow-up of Stethographically Recorded Functional Murmurs in Children.

6. INVESTIGATORS
Dr. Carl J. Marienfeld
Dr. Bert Boone
Miss Marie Nordsieck

7. LOCATION OF PROJECT
Hagerstown, Maryland

8. DATE PROJECT INITIATED
April 1958

9. OBJECTIVES
To make a longitudinal evaluation using stethograms, electrocardiograms, and X-rays taken 20 years ago among Hagerstown, Maryland, school children. If internists are correct and functional murmurs are significant, they should still be present and associated with other manifestations of heart disease in the group of persons followed. If, on the other hand, the pediatricians are right and functional murmurs are normal for children and adolescents, then the murmurs should have largely disappeared, and not more than 5 cases of heart disease should be observed (based on an expected prevalence of 2%* in the general population with an allowance for sampling variation).

10. PRINCIPAL RESULTS
Of the 158 children with loud but apparently functional murmurs who were selected from among 4,000 who had phonocardiographs in 1938, 100 were located, and 97 have had examinations which included physical examination, laboratory studies, X-ray, phonocardiogram and electrocardiogram. All of these readings have been completed. Analysis will begin after September 1, 1959, and the work should be completed by December 31, 1959.

11. PUBLICATIONS
None

*Based on 3,300,000 selective service examinations of men between 18 and 37 years of age; conducted from 1940 through 1952.

1. Living
Special needs Seaview

2. Problem
Bears Mistook Canyon

3. Problem
Temperature Relative to statistically Recorded Numerical

4. Problem
Dog-10-20

5. Problem
Injuries In Children.

6. Duplication
Dr. Carl J. Hartsdale
Dr. Mark Moore
Miss Sheila Pennington

7. Location of Problem
Engebretson, Mockjens

8. Duplication
To make a longitudinal evaluation using electrocardiograms, dictaphones, surgical
gloves, and X-rays taken 20 years ago among respondents, measure the alignment
of bones. It includes an internal incision which makes manipulation
children. Is Inscentive at Present and Increased which other manipulation
they should still be present and increase inflated. (B) or Only one Point.
of Heart Disease in the group of persons inflated Increase or Report for children.
the pedicardiums are right and Increase Increase or Report disappearing and Not
and adolescents. Then the persons should be observed (Based on an
more than 3 cases of Heart disease should be reported with an injection
reported prevalence of DIH in the western population with an injection
the smoking variation).

10. Problem Prevalence
In the 150 children with lung per apparently functional problem
who were infected, and or have and compensations which included physical
200 were infected, X-ray, pneumonia/pneumonia and electro-
cardiogram. Laboratory studies have been completed. Analysis of
Joslin Alter Schneider J, 1922, and the lungs should be completed by
December 31, 1925.

11. Duplication
Dyne

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-21

5. PROJECT TITLE
The Use of an Electronic Computer as a Diagnostic Aid.

6. INVESTIGATOR
Dr. Arthur E. Rikli

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
December 1957

9. OBJECTIVES
To explore how an electronic computer might serve as a diagnostic aid to physicians.

10. PRINCIPAL RESULTS
The first phase of the work plan, the feasibility study, has been completed. Forty-five data points have been manually measured for 15 normal and 15 pathological subjects. IBM punch cards have been prepared. Tabulations of data are almost completed. Data analysis will follow.

11. PUBLICATIONS
None

SUPPLEMENT TO NASA CONTRACT OR PROGRESS

DATE: August 5, 1970

1. AUTHOR(S)
   Special Media Services

2. AGENCY

3. PERFORMING ORGANIZATION
   Space Library Control

4. PERFORMING ORGANIZATION REPORT NUMBER
   SMS-SD-71

5. PROJECT TITLE
   The Use of an Electronic Computer as a Diagnostic Aid.

6. SPONSORING AGENCY
   Dr. Arthur D. Kidd

7. LOCATION OF COMPANY
   Washington, D. C.

8. DATE PUBLICATION AUTHORIZED
   December 1957

9. ABSTRACT
   To explore how an electronic computer might serve as a diagnostic aid to physicians.

10. PUBLICATION RESTRICTIONS
    The first phase of the work pilot, the feasibility study, has been completed. Forty-five terms have been successfully mechanized for 15 mental and 15 psychological symptoms. (5) panel cards have been prepared. Technicians of Omega Med Center complete. Data analysis will follow.

11. PUBLICATIONS
    None

PROJECT CONTROL RECORD

SECTION I PROJECT NO. HD-24

CONTRACT NO._____

1. TITLE Development of Methods for Assessing the Adequacy of Cardiovascular Disease Mortality Reporting
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSONS (HDCP) Herbert Sauer (Jeremiah Stamler (Iwao Moriyama, Chief, Mortality Analysis, NOVS
5. LOCATION HDCP, Washington, D. C.
6. RESPONSIBLE PERSON (Field)
7. ADDRESS (Mailing)
8. COOPERATING AGENCIES: National Office of Vital Statistics
9. DATE (a) Contract Approved: (b) Project Started: 9/58 (c) Project Completed:

SECTION II

1. BACKGROUND: Objective: To outline techniques for assessing the accuracy of cardiovascular death rates, particularly in groups in which routine autopsy verification of cause of death is not available. A minimum goal is to develop standards from which inferences could be drawn regarding differences in mortality rates at least to the degree that: (a) the differences in the specified rates are great enough and meet specific tests in a way that they most probably represent real differences even though proof may be lacking as to the exact magnitude of the difference; (b) there is sufficient information to determine whether the differences are real; (c) These differences are consistent with specified hypotheses that the differences easily may be due entirely to differences in classification of cause of death or other data collection methods.

REPORT NO. _____ PROJECT NO. 88-26

DOCUMENT NO. _____

TITLE: Development of Methods for Defining the Appearance of Crystalline Materials

Alliance Mortgage Corporation

STATE Arizona

SECTION Administration

CITY/TOWN Phoenix

PERSON(S) Robert Moore (Project Director, Security Mortgage Co.)
Theresa Lindsey, Secretary

ADDRESS 1234 Main, #203

TELEPHONE(S) 602-555-1212

CONTRACTOR(S) National Office of Title Insurance

ADDRESS

TELEPHONE(S)

LOCATION Must Coordinate by 5

PERSON(S) (a) Purchase (b) Int. Prof. (c) Employee

MAIL Approved: Returned Date Completed:

(DELETE 1)

Procedures: Objectives: To evaluate principles for processing the accuracy of appraisal data collection methods.

Abstract: Determinations are considered with specified appropriateness that the difficulties in determination to discerning whether the differences are real; (c) these differences may be due entirely to differences in classification of cause of error or other data collection methods.

PROJECT CONTROL RECORD

SECTION I PROJECT NO. SHS-HD-26

CONTRACT NO.

1. TITLE Epidemiology of Cardiovascular Mortality in the Carolinas and Georgia
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP) Herbert Sauer
5. LOCATION HDCP, Washington, D.C. Raleigh, North Carolina and Columbia, S. Car., Atlanta, Georgia
6. RESPONSIBLE PERSON (Field) Full Name: Joseph Conte Title: Public Health Representative
7. ADDRESS (Mailing) Atlanta, Georgia
8. COOPERATING AGENCIES: National Office of Vital Statistics, State Health Departments, PHS - Regional Offices III and IV.
9. DATE (a) Contract Approved: (b) Project Started: 4/59 (c) Project Completed

SECTION II

1. BACKGROUND: Objective: To determine if the high cardiovascular mortality rates for the Carolinas and Georgia are accurate, and to determine if the extreme contrasts in rates for these States are real.

PROJECT CONTROL RECORD

PROJECT NO. DRS-HB-20

CONTRACT NO.

1. TITLE Breakdown of Conditioned Mobility in the Deer Mouse

Domain

2. KEYS: Research

3. DESCRIPTION Microcomputer

4. RESPONSIBLE

5. LOCATION Psychology Bldg. Columbia, S. Car., Williams, Upcraig

Psych

Psychology Dept.

6. RESPONSIBLE Prof. Jones

Public Health Representative

7. ASSIGNED

Graduate Student

8. CONTRACTING AGENCY National Office of Vital Statistics, Grand Rapids

Research, PHS - Purchase Order No. 121 ACC 24

9. DATE (a) Contract (b) Project (c) Project

Awarded Expires Completed

10. RECOMMENDED Objectives: To determine if the high cardiovascular mortality rate in Grand Rapids for the Caucasian male can be explained by a genetic component.

Methods: Experimental manipulation in mouse model.

DESCRIPTION

PROJECT CONTROL RECORD

PROJECT NO. HD-27

SECTION I

CONTRACT NO.

1. TITLE Heart Disease Screening - Computer Evaluation Method
2. TYPE Special Project
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP) Cesar Caceres, M.D. and Sidney Abraham
5. LOCATION HDCP - Washington, D. C.
6. RESPONSIBLE PERSON (Field)
7. ADDRESS (Mailing
8. COOPERATING AGENCIES:
9. DATE (a) Contract Approved: (b) Project Started: 1958 (c) Project Completed:

SECTION II

1. BACKGROUND: Since 1958 the Heart Disease Control Program has been exploring the use of electronic computers for screening for heart disease, by feeding multi-test data into the machine and programing it in such a way that objective data will be classified into clinically significant groups of observations. The use of computers for reading ECG tracings is under study.
   A committee was formed in 1958 to investigate the feasibility and cost of such a project. The above employees have been assigned to carry ahead this investigation. (1960)

2. METHODOLOGY: Four contracts and/or agreements are presently let regarding specific portions of these investigations and more contracts with other electronic computer manufacturers will probably be let during the coming year.

3. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

   a. HDCP (Contract) Amount: $115,297

4. BOOK (Part, etc.) _____ VOLUME: _____

5. SUBORDINATE ASSOCIATION: _____

6. SECONDARY SUBDIVISION _____

7. Write a brief abstract or description of the work. Include the purpose of the study, the principal findings, and the significance of the results. If the study is not complete, indicate what has been accomplished and what remains to be done.

ABSTRACT: Some conventions used in typesetting are primarily for aesthetic purposes, while others are more functional in nature. Typographers have developed a number of rules governing the use of punctuation marks, capitalization, and spacing. Many of these rules are not universally agreed upon, and even when they are, they are frequently violated. This article examines the most common typographical conventions and suggests a practical approach to their use.

8. Write a brief abstract or description of the work. Include the purpose of the study, the principal findings, and the significance of the results. If the study is not complete, indicate what has been accomplished and what remains to be done.

ABSTRACT: Some conventions used in typesetting are primarily for aesthetic purposes, while others are more functional in nature. Typographers have developed a number of rules governing the use of punctuation marks, capitalization, and spacing. Many of these rules are not universally agreed upon, and even when they are, they are frequently violated. This article examines the most common typographical conventions and suggests a practical approach to their use.

9. DATE: (a) Received _____ (b) Revised _____ (c) Accepted _____

Approved _____, Director _____ (Signature)

10. SUPPLEMENTARY ASSOCIATES: _____

11. (Specific) _____

12. ADDRESS _____

13. EDITION (Title) _____

14. SPONSORSHIP _____

15. LOCATION _____ DOCK - Shipbuilding I.C. _____

PERIOD (SHIP) _____ Ocean Voyages, Ports and Sights Abroad _____

16. DISTRIBUTION/ _____

AVAILABILITY _____ Abbreviated _____

17. _____ TITLE Special Issues _____

18. _____ TITLE Short Stories Parameters, Systems to Environment Analysis _____

19. _____

Date _____

Signature _____ Date Signed _____

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1960

1. DIVISION: Special Health Services
2. BRANCH: Heart Disease Control Program
3. SECTION: Technical Services
4. LOCATION OF PROJECT:

   Washington, D. C.

5. DIVISION PROJECT NUMBER-SHS-HD-28

   DIVISION CONTRACT NO. SAph 75200

6. DATE WHEN INITIATED-6/15/60

7. PROJECT TITLE:

   Telephone and radio transmission of electrocardiogram

8. INVESTIGATORS:

   Dr. Harvey Proctor
   Dr. Norman Barr
   Dr. M. G. Criscitiello
   Dr. Cesar A. Caceres
   Mr. Sidney Abraham

9. PARTICIPATING ORGANIZATIONS:

   Georgetown University Hospital
   U. S. Public Health Service, Heart Disease Control Program

10. To investigate the possibility of expediting transmission of electrocardiograms from subject at a distal point to electrocardiographer by telephone and radio. The centrally located Heart Station in Georgetown University Hospital will return the interpreted electrocardiograms to the physician before he has completed his rounds or consultations. This method would provide a better utilization of cardiographer's time with the institution of a constant and regular flow of electrocardiograms from several sources in the Hospital. Potentially, the method would bring to each physician in his office the equivolent of the diagnostic aids available in large medical centers.

    Georgetown University Hospital electrocardiographic work done at the hospital to provide a sufficient number of tracings in a given day to enable evaluation of the efficiency of rapid transmission. Apparatus will be provided for the remote transmission of electrocardiographic tracings from the bedside of the patient to the heart station. The subject is attached by electrodes to a standard electrocardiographic machine. The signal on the standard electrocardiographic machine is recorded as usual for the use of the individual physician interested in the patient, but simultaneously this signal is modulated in a special

unit attached to a telephone or radio by which signals would be transferred to the central heart station. The signal would be demodulated by a special unit attached to the standard electrocardiographic machine. On termination of reading by electrocardiographer, a telephone call back would be made. The system of bedside recording in parallel with the new technique provides two tracings so that the standard tracing can be compared with the tracing obtained directly in the heart station. Thus, the validation of the technique will be made by comparing the fidelity of remote transmission. This will indicate the degree in which an electrical device accurately reproduces its effect. Other factors to be evaluated are cost- and time-saving features of the telephone or radio system contrasted with the conventional method of recording electrocardiograms to a heart station.

unit extended to a telephone or radio by which signals would be transferred to the control board station. The signal would be decoded by a special unit attached to the standard electromechanical or electronic telephone. In termination of receipt by electromechanical or electronic phone call back would be made. The system of dealing accordingly is peculiar with three functions providing two routings to each the standard routing can be changed with the routing obtained directly in the house station. Thus, the validation of the combine will be made by comparing the fidelity of remote transmission. This will indicate the degree to which an electrical device operate by reproduces its effects. Other factors to be evaluated are real and time-saving features of the telephone or radio system connected with for communications method of connecting electronic users to a local station.

PROJECT CONTROL RECORD

PROJECT NO. HD-29

SECTION I

CONTRACT NO. 73486

1. TITLE Development of Instruments and Protocol for Study of Stress as Related to Coronary Artery Disease and Hypertension.
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP) Leonard Bornstein, M.D.
5. LOCATION Lansing, Michigan
6. RESPONSIBLE PERSON (Field) Dr. Lawrence Schlessinger
7. ADDRESS (Mailing)
8. COOPERATING AGENCIES:
9. DATE (a) Contract Approved: (b) Project Started: 11/1/59 (c) Project Completed:

SECTION II

1. BACKGROUND: One of the factors currently under suspicion in the development of coronary heart disease is psychological stress involving the pressures inflicted on the individual by his environment. Little is known about the degree to which this variable operates.

2. METHODOLOGY: Design: 1. A series of questionnaires to record and measure levels of psychological stress; 2. protocol for validating stress measurements applied to information recorded in the questionnaires. Application of the above to a population group which will be screened and followed for the presence or development of coronary artery disease or hypertension.

3. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

a. HDCP (Contract) Amount: $1,500.00

PROJECT CONTROL RECORD

SECTION X

CONTRACT NO. _____ PROJECT NO. 30-22

1. TITLE: Development of Performance and Procedures for Study of Research on Contract Effort Planned Studies and Investigations.
2. TYPE: Research
3. DIRECTION: Alternate(Personal
4. ADDRESS:
PERSON (CHIEF): Leonard Bourne, Jr., M.D.
5. DEPARTMENT: Industrial Medicine
6. INSTITUTION: Research Institute
ADDRESS: 75 Lexington Parkway
PERSON (CHIEF): Dr. Lexington Parkwayington
7. DATE: (a) Commenced: (b) Projected (c) Projected
Approved: Started: 6/1/52 Completed:
8. SPONSORING AGENCIES:
(Name)
9. ADDRESS:
10. Brief Description: Aim of the Research originally under Direction in the Laboratory of Personnel Health directed by his Assistant. Study is being expanded to include the Research on this Contract.

11. RECOMMENDED: Average 1. A Section of Industrial Medicine to Record and Measure Levels of Occupational Stress. 2. Personal Interviewing Should Be Conducted to Discussion Research in the Psychosomatics. Application of the above to a Population Group which will be Processed and Information for the Purpose of Developing of Secondary Among Diseases of Depression.

12. ACTIVITY(S) DURATION:

13. SUPERVISING AGENCIES:

14. FUND (Contract) Amount: $1,500.00

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1960

1. DIVISION: Special Health Services

2. BRANCH: Heart Disease Control Program

3. SECTION: Rheumatic and Congenital Heart Diseases

4. LOCATION OF PROJECT: Washington, D. C.

5. DIVISION PROJECT NUMBER
SHS-HD-30

6. DATE WHEN INITIATED
January 1960

7. PROJECT TITLE: College Rheumatic Fever Motivational Study

8. INVESTIGATORS: Dr. Carl Marienfeld
Dr. Leonard Syme
Miss Margaret Evans
Mr. Eldon Kronewitter
Mr. William Rouse

9. PARTICIPATING ORGANIZATIONS: 11 Universities and Colleges

10. OBJECTIVE AND RESEARCH PLAN: To determine why students having a definite history of rheumatic fever and/or rheumatic heart disease are or are not on prophylaxis.

Interviews were conducted with approximately 400 students with a positive history of rheumatic fever and rheumatic heart disease. These students were chosen from a selected sample of all colleges and universities to give a geographic distribution. Previous data had shown that less than 11% of those students with positive histories of rheumatic fever and rheumatic heart disease were currently on prophylaxis, and that only 50% had ever been placed on a prophylactic regimen. This study is concerned with the student's knowledge of his illness and the motivation for his prophylactic status. Data are currently being analyzed.

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1960

1. DIVISION: Special Health Services

2. BRANCH: Heart Disease Control Program

3. SECTION: Rheumatic and Congenital Heart Diseases

4. LOCATION OF PROJECT: Chicago, Illinois

5. DIVISION PROJECT NUMBER
SHS-HD-31

DIVISION CONTRACT NUMBER
SAph 74176

6. DATE WHEN INITIATED
March 15, 1960

7. PROJECT TITLE: Research on Identification of Group A Beta Hemolytic Streptococci from Direct Smears

8. INVESTIGATORS: Dr. Alan Siegel, Chicago
Mr. Harold K. Wood

9. PARTICIPATING ORGANIZATIONS: Children's Memorial Hospital

10. OBJECTIVE AND RESEARCH PLAN: A previous pilot study indicated that the immediate fluorescent of a smear from a direct throat swab failed to correlate well with the conventional grouping method. Purpose is to compare the conventional precipitin method of grouping beta hemolytic streptococci with the fluorescent antibody technique for identifying group A streptococci from direct throat swabs.

The specific points of comparison include the accuracy of this method and the practicality of application to a routine bacteriology laboratory.

PRODUCTION SCHEDULING WORKSHEET

Date: August 5, 1960

DEPARTMENT: Special Metals Services 3. INVERSION PROJECT NUMBER
                                     SMS-60-31

BRANCH: Heavy Stamping Contract Program

SECTION: Mechanic and Computing Heavy Fixtures RAJU MATTA

                                     PURCHASING CONTRACT NUMBER

LOCATION OF PURCHASER: Chicago, Illinois 6. Main Unit Shipped
                                     March 15, 1960

ACCOUNT DATA: Assessed on Classification of Grade A Data Manually
                Stockpiled from Other Areas

EMPLOYEE NAME: Dr. Alan Kingsley Ohlenge
               Mr. Harold T. Ford

TAPE RECORDING ORGANIZATION: Multichannel Magnetic Journal

OBJECTIVE AND RESPONSE PLAN: A derivative yield study indicated that the chocolate flavorcenter of a mocha cream & fruit cherry, each flavor coated with the conventional pouring method. Purpose is to compare the conventional pouring method of dropping bare hopolyzic drops & intercepted from dairy cheese sample.

The operation of purchase of corporation involves the accuracy of this method and the practicality of application to a complex accounting industry.

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1960

1. DIVISION: Special Health Services

2. BRANCH: Heart Disease Control Program

3. SECTION: Rheumatic and Congenital Heart Diseases

4. LOCATION OF PROJECT: Bismarck, North Dakota
Mandan, North Dakota

5. DIVISION PROJECT NUMBER
SHS-HD-32

DIVISION CONTRACT NUMBER
SAph 73518

6. DATE WHEN INITIATED
September 18, 1959

7. PROJECT TITLE: North Dakota Streptococcal Study

8. INVESTIGATORS: Dr. Walter Pretorius
Dr. W. C. Nicholas
Dr. Carl Marienfeld

9. PARTICIPATING ORGANIZATIONS: North Dakota State Department of Health

10. OBJECTIVE AND RESEARCH PLAN: The epidemiology of group A streptococci and rheumatic fever in a random school population is being studied to determine the relationship between the occurrence of the infection and the incidence of the disease. Further information furnished will be the relationship of signs and symptoms to streptococcal occurrence, and the curve of this infection incidence in grade and junior high school.

Publication Information

Date: August 5, 1993

3. Publication Number
   ISSN-0000-00

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Annual Report of Disease Control Centers

Monthly Morbidity and Communicable Disease Digest

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Circulation Manager: Jim Boyd

Publication Office: Department of Health
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Bismarck, North Dakota 58505-0200

Publication Number: 3

Publication Date: August 5, 1993

Publication Title: North Dakota Disease Control Report

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1960

1. DIVISION: Special Health Services

2. BRANCH: Heart Disease Control Program

5. DIVISION PROJECT NUMBER
SHS-HD-33

DIVISION CONTRACT NUMBER
SAph 73797

4. LOCATION OF PROJECT: Colorado

6. DATE WHEN INITIATED
March 24, 1960

7. PROJECT TITLE: Incidence of Streptococcal Infections and Rheumatic Fever

8. INVESTIGATOR: Dr. Carl Marienfeld

9. PARTICIPATING ORGANIZATION: State of Colorado Department of Public Health

10. OBJECTIVE AND RESEARCH PLAN: To furnish information on the scope and etiology of streptococcal infections in the State of Colorado during FY 1960, and their associations with reported cases of rheumatic fever additionally, the data on the number of cases administered prophylaxis, the kind and the effectiveness of prophylaxis will be furnished.

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1960

1. DIVISION: Special Health Services

5. DIVISION PROJECT NUMBER
SHS-HD-34

2. BRANCH: Heart Disease Control Program

DIVISION CONTRACT NUMBER
SAph 73533

3. SECTION: Rheumatic and Congenital Heart Diseases

4. LOCATION OF PROJECT: New York City

6. DATE WHEN INITIATED
Oct. 2, 1959

7. PROJECT TITLE: Rheumatic Fever Recurrence Rates

8. INVESTIGATORS: Dr. Eugenie F. Doyle
Dr. David Brand

9. PARTICIPATING ORGANIZATION: New York University College of Medicine

10. OBJECTIVE AND RESEARCH PLAN: To investigate the experience of the Adult Cardiac Clinic at New York University-Bellevue Medical Center in the recurrence rates of acute rheumatic fever among patients with and without penicillin prophylaxis. The investigator proposes to supply data on changing morbidity and mortality over the years, as well.

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1904

1. KEYWORDS: Special Metals Brewing

2. BRANCH: Heart Disease Control Program

3. DIVISION PROJECT NUMBER
   SHA-10-34

4. LOCATION OF PROJECT: New York City

5. DESCRIPTION: Rheumatic and Pneumatical Heart Diseases

6. INVESTIGATORS: Dr. Benjamin C. Hoyle
   Dr. Myrle Pound

7. PROJECT TITLE: Spinnable Cheese Decorations Wines

8. DATE WORK INITIATED
   Oct. 3, 1939

9. PARTICIPATING ORGANIZATIONS: New York University College of Medicine

10. COOPERATIVE AGREEMENTS: The Investigation the operations at the Public
    Coastal Clinic at Southern University-Division Medical College in the
    treatment only. At route Pensacola Ferry among patients to supply data
    for Dependents Aptitudes. The correlation between the degree, as well.

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1960

1. DIVISION: Special Health Services

2. BRANCH: Heart Disease Control Program

3. SECTION: Rheumatic and Congenital Heart Diseases

4. LOCATION OF PROJECT: Rockville, Md.

5. DIVISION PROJECT NUMBER
SHS-HD-35

DIVISION CONTRACT NUMBER
SAph 73544

6. DATE WHEN INITIATED
Sept. 25, 1959

7. PROJECT TITLE: Field Study of Fluorescent Antibody Technique

8. INVESTIGATORS: Dr. William J. Peeples
Mr. Dan Spielman
Mr. Harold K. Wood

9. PARTICIPATING ORGANIZATION: Department of Public Health, Montgomery County, Maryland

10. OBJECTIVE AND RESEARCH PLAN: To provide the Public Health Service with knowledge of the problems and solutions arising from the practical application of the fluorescent antibody technique to streptococcus identification. The Montgomery County program is to make this diagnostic service available to all the private physicians in the county. The information of the number of cases and the costs, and a full evaluation of the administration and technical methods used in making the technique available through local health department auspices, will be furnished by the contractor. This was also a validating study to compare the fluorescent antibody technique with the conventional laboratory method.

RESEARCH PROJECT DESCRIPTION

Date: August 7, 1980

[illegible]: Special Health Problems [illegible] 6. DURATION EXPECT NEEDED:
384-20-04

[illegible]: Acute Disease Control Program

[illegible]: Economic and Applied Nurse Diseases Grant 17584

[illegible] OF SCHOLAR: Nonspecific [illegible] 9. DATE WORK INITIATED
Sept. 27, 1979

[illegible]: Field Studies of Circumscure Morphology Problems

[illegible]: Dr. William D. Forgue
Mr. Dean M. [illegible]
Mr. Donald E. Wood

[illegible] ORGANIZATION: [illegible] of Public Health. Montgomery
[illegible], Maryland

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PROJECT CONTROL RECORD

PROJECT NO. HD-36

SECTION I

CONTRACT NO. 73824

1. TITLE Coronary Artery Disease among Seventh-day Adventists in California
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP)
5. LOCATION Loma Linda, California
6. RESPONSIBLE PERSONS (Field) Richard T. Walden, M.D. and Frank R. Lemon, M.D.
7. ADDRESS College of Medical Evangelists

(Mailing)	City	State
	Loma Linda	California

8. COOPERATING AGENCIES: College of Medical Evangelist of Loma Linda
9. DATE (a) Contract Approved (b) Project Started: 4/15/60 (c) Project Completed:

SECTION II

1. BACKGROUND: A previous study indicated that Seventh-Day Adventist may have a significantly different experience with coronary artery disease than non-Seventh-Day Adventist. Seventh-Day Adventists are reported to be a virtually non-smoking, non-drinking population with dietary and other habits substantially different from most of the rest of the general population. The objective of this study is to obtain a comparison of mortality from coronary artery disease among Seventh-Day Adventist in California and the total coronary artery disease mortality for the State of California.

2. METHODOLOGY: 47,611 Seventh-Day Adventists in California were enrolled in a retrospective and prospective cancer study in 1958, including continuous follow-up of the group for mortality experience by matching for deaths against the State Vital Record Index. The existing records and machinery will be used to obtain the mortality experience from coronary artery disease 1959-1961 for this group. If significant differences are found, a larger epidemiological study will follow.

3. ESTIMATED DURATION: 4/15/60 - 3/30/62

4. SUPPORTING AGENCIES:

a. HDCP (Contract) Amount: $19,755.00

Project Control Number

Project No. 80-16

Contract No. 79124

Title: Comparative Archery Classes among Taxpayer-Only Adult Males in California

Type of Report: Preliminary

Author(s): Alphonse Clarence Jones, Linda Jones, and Frank R. Lyon, M.D.

Performing Organization: Jones Linda Institute

Address: 1 Jones Lane, Jonesville, California

Sponsoring Agency: (1) Director of Jones Linda Institute

Abstract: A previous study conducted from Seventy-Day Adventist and Non-Seventh-Day Adventist archery classes with secondary archery classes has been reported to be significantly different from that of the general population. The objective of this study is to find out if the general and other archery classes would be similar to those of the Seventh-Day Adventist archery classes. Data were collected from the state of California and other states. Comparisons were made between the Seventh-Day Adventists and non-Seventh-Day Adventists. Significant differences were found in the following areas: [illegible] Seventh-Day Adventists in California have a longer life expectancy than the general population. The objective and subjective methods of [illegible] were used. The findings indicate that the Seventh-Day Adventists in California have a longer life expectancy than the general population. The objective and subjective methods of [illegible] were used. The findings indicate that the Seventh-Day Adventists in California have a longer life expectancy than the general population.

Approved: (1) Director

Submitted: 12/15/80

Completed: (2) Project Director

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1960

1. DIVISION: Special Health Services

5. DIVISION PROJECT NUMBER
SHS-HD-37

2. BRANCH: Heart Disease Control Program

3. SECTION: Rheumatic and Congenital Heart Diseases

4. LOCATION OF PROJECT: Twenty-six States, Puerto Rico, and Communicable Disease Center, Atlanta, Georgia

7. PROJECT TITLE: Fluorescent Antibody Training and Technique Evaluation

8. INVESTIGATORS: Dr. David Brand
Mr. Ken Wood

9. PARTICIPATING ORGANIZATIONS: Twenty-six States and Puerto Rico--

Alabama	Connecticut	Iowa	New Jersey	Oregon	Texas
Alaska	Florida	Kentucky	New York	Pennsylvania	Vermont
California	Idaho	Louisiana	North Carolina	Rhode Island	Washington
Colorado	Illinois	Minnesota	Oklahoma	Tennessee	W. Va.
					Wisconsin

10. OBJECTIVE AND RESEARCH PLAN: One technician from each State was trained in the fluorescent antibody technique by the Communicable Disease Center. Composite validating results from 11 States show the correlation of the fluorescent antibody technique, as compared with conventional culturing techniques, to be: sensitivity 90.79% and specificity 97.57%.

PROJECT CONTROL RECORD

PROJECT NO. HD-38

SECTION I

CONTRACT NO. 75449

1. TITLE Application of Socio-psychological Methods in Analyzing Problems of Rehabilitation of Disabled Stroke Patients.
2. TYPE Research - Demonstration
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSONS (HDCP) 1. Al Rhudy, 2. Arleigh Sawyer
5. LOCATION Lansing, Michigan
6. RESPONSIBLE PERSON (Field)
7. ADDRESS (Mailing)
8. COOPERATING AGENCIES: Michigan State Department of Health
9. DATE (a) Contract Approved: (b) Project Started: 5/18/60 (c) Project Completed:

SECTION II

1. BACKGROUND: The objective of the project is to demonstrate the application of socio-psychological methods in analyzing problemsof rehabilitation of disabled stroke patients by: (1) Determining the problems of rehabilitation in a complete rehabilitation center, (2) translating the problems into generally applicable theoretical problems, and (3) outlining studies aimed at clarifying and solving these problems.

2. METHODOLOGY:

3. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

a. HDCP (Contract) Amount: $2,950.00

1. Name (Print) _____ Phone: _____

2. Department _____

3. Position Title _____

4. Briefly describe your activity that _____

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Briefly describe your activity that _____

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PROJECT CONTROL RECORD

PROJECT NO. HD-39

SECTION I

CONTRACT NO. _____

1. TITLE Cholesterol Standardization Program
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP)
5. LOCATION Atlanta, Georgia
6. RESPONSIBLE PERSON (Field) Gerald Cooper, M.D.
7. ADDRESS (Mailing) Communicable Disease Center
   Atlanta, Georgia
8. COOPERATING AGENCIES: Communicable Disease Center - PHS
9. DATE (a) Contract Approved: (b) Project Started: 9/59 (c) Project Completed:

SECTION II

1. BACKGROUND: Objective: To determine the effects of shipment or specimen on blood cholesterol levels.

2. METHODOLOGY: Specimens of blood, drawn and tested in the Communicable Disease Center Laboratory, will be shipped to four longitudinal laboratories for examination. The results will be compared with those of the Communicable Disease Center.

3. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

   a. HDCP (Contract) Amount: $3,000.00

CLINIC CONSENT RECORD

PATIENT NO. MH-13

DECISION C

SIGNATURE NO.

TITLE Cholesterol Standardization Program

TYPE Diagnostic

ACTION Allergoselection

REQUIRED

SIGNATURE (LEGAL)

DESIGN Adiatax Complex

SIGNATURE

TERMS (Limited) Mental Capacity Only

CONSENT Consentable Injury Donor

(medium)

ADVANCE (Service)

SUPERVISOR ADDRESS: Consentable (Up-to-Date Center - TMC)

DATE (/) Guardian (P) Patient (O) Physician

Additional: 0 Electric 3/30 Consultant

BACKGROUND: Clinical: To decrease risk related to elevated or Aggressive Behavioral Malfunctions Incident.

MEDICATION: Physicians or Staff, always not accept in the Consultant Clinic Service. Clinic Service Procedure . To minimum to time Staff Clinical Physicians for evaluation, the results - if or consent with Clinic or Consultant Clinic Service Procedure.

9. NOTE (Continued) Answer 3/30/05

PROJECT CONTROL RECORD

PROJECT NO. HD-40

SECTION I

CONTRACT NO. 73780

1. TITLE Retrospective Study of Survivorship among "Temporary Disability Program" Claimants Diagnosed as Artereosclerotic Heart Disease.
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSONS (HDCP) Carl Marienfeld, M.D. and Edith Jungblut
5. LOCATION Providence, Rhode Island
6. RESPONSIBLE PERSON (Field)
7. ADDRESS (Mailing) No. 24 Mason Street; City Providence; State Rhode Island
8. COOPERATING AGENCIES: Rhode Island Department of Employment Security, Temporary Disability Program
9. DATE (a) Contract Approved: (b) Project Started: 3/10/60 (c) Project Completed: 4/1/60

SECTION II

1. BACKGROUND: Objective: To describe the natural history of artereosclerotic heart disease in a working population.

2. METHODOLOGY: Records from the files of the Temporary Disability Program will provide information on the history of 887 diagnosed cases of coronary heart disease. The information will be transferred to punch cards and matched to death certificates to provide mortality data for the entire cohort. Punch card listings and supplemental data on the R. I. labor force and the Temporary Disability Insurance covered population will be given to the Heart Disease Control Program for Study and analysis.

3. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

   a. HCDP (Contract) Amount: $2,000.00

Employee Contact Record

Section I Contract No. 77750 Project No. JD-00

[illegible] Prospective Study of Progressive Pneumoconiosis in Coal Miners

[illegible] (Johnstown) Diagnosed in Asymptomatic Heart Disease

Type Research

Section A(Dependents)

Spouse (Ref) Mrs. Jo Ann Edith Josephine

Address No. Jo Sweet Street Steve

(Position (Title))

Employer City Rhode Island

Employer's Address: Rhode Island Department of Employment Security

Temporary Disability Program (b) Project (c) Project

Date (a) Compare Date Ind. 2/M/60 Of Refresh/1/60

Approved

Section II

Background: Collection: To observe the normal Sleep of Afternoon(Night)

Note Disease I. 1 Morning completion.

Methodology: Records from the files of the Temporary Disability Program

will provide information on the history of [illegible] diagnosed cases of pneumoconiosis

heart disease. The interviews will be conducted to permit correlation

correlated to durch complications to Tremolis activity data on the X. Ray Index

color. Read, read lighting and supplemental data on the X. Ray Index

and the Temporary Disability Insurance program for study and analysis.

Type Report Disease Control Programs for Study and Analysis

Section A, Authorized Duplicate

Section B, Duplicate Authorized

Section C, None (Contra-C)

Section D, [illegible]

PROJECT CONTROL RECORD

PROJECT NO. HD-41

SECTION I

CONTRACT NO. 73800

1. TITLE Congestive Heart Failure in a Community
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP)
5. LOCATION City: Framingham State: Massachusetts
6. RESPONSIBLE PERSON (Field) Julian Litten, M. D.
7. ADDRESS (Mailing) No. Street
8. COOPERATING AGENCIES:
9. DATE (a) Contract Approved: (b) Project Started: 3/23/60 (c) Project Completed:

SECTION II

1. BACKGROUND: To use available data to determine the prevalence and incidence of congestive heart failure in a community; identify factors preceeding the development of congestive failure and determine the prognosis and cost of such illness.

2. METHODOLOGY: Using the records of the Framingham, Massachusetts longitudinal study of 4,500 residents, examined and followed since 1948, new criteria will be applied to study congestive heart failure in this group.

3. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

a. HDCP (Contract) Amount: $5,000.00

PROJECT CONTROL RECORD

PROJECT NO. HD-42

SECTION I

CONTRACT NO. 75323

1. TITLE Bryn Mawr Stroke Study
2. TYPE Research - Social
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSONS (HDCP) (1) Al Rhudy, (2) Arleigh Sawyer
5. LOCATION Delaware County, Pennsylvania
6. RESPONSIBLE PERSON (Field) Dr. Katherine Lower
7. ADDRESS (Mailing) No. Street City State
8. COOPERATING AGENCIES: 1. Pennsylvania State Department of Health 2. Bryn Mawr College, School of Social Work
9. DATE (a) Contract Approved: (b) Project Started: 6/16/60 (c) Project Completed:

SECTION II

1. BACKGROUND: Delaware County, Pennsylvania is conducting a stroke program which includes the education of professional workers and public, home care of patients, and full use of community resources. The objective of the study is to attempt to identify social factors conditioning the convalescence of stroke patients.

2. METHODOLOGY: A selected group of patients receiving the services of a social agency will be compared with a similar group who do not receive these services. The study will be carried out by the faculty of the Bryn Mawr College, School of Social Work. Data will be collected by interview and tabulated by machine. The study will attempt to identify the optimal social welfare techniques for restoring stroke patients to maximum community usefulness and obviate the need for public institutional care.

3. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

   a. HDCP (Contract) Amount: $7,500.00

PROJECT CONTROL RECORD

PROJECT NO. HD-43

SECTION I

CONTRACT NO. 73821

1. TITLE Determination of Prevalence of Clinically Diagnosed Coronary Disease.
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSONS (HDCP) 1. Marjorie Cantoni, 2. Herbert Sauer
5. LOCATION Evans and Bullock Counties, Georgia
6. RESPONSIBLE PERSON (FIELD) Curtis G. Hames, M.D.
7. ADDRESS (Mailing) Evans County Health Department, Claxton, Georgia
8. COOPERATING AGENCIES: Georgia State Department of Public Health
   Hames Clinic, Claxton, Georgia
9. DATE (a) Contract Approved: (b) Project Started: 4/20/60 (c) Project Completed:

SECTION II

1. BACKGROUND: Objective: To provide baseline data for determining prevalence of clinical coronary disease in age-adjusted, race and sex, specific groups for 3,000 persons in Evans and Bullock Counties, Georgia.

2. METHODOLOGY: Selected physical and laboratory examinations.

3. ESTIMATED DURATION: 6/30/60

4. SUPPORTING AGENCIES:

   a. HDCP (Contract) Amount: $32,000.00

PROJECT CONTROL RECORD

PROJECT NO. HD-44
73520

SECTION I

CONTRACT NO. 73762

1. TITLE Resume and Evaluation of the Georgia Stroke Program

2. TYPE Demonstration

3. SECTION Atherosclerosis

4. RESPONSIBLE PERSON (HDCP) Arleigh Sawyer

5. LOCATION Georgia

6. RESPONSIBLE PERSON (Field) Joseph E. Gahimer, M. D.

7. ADDRESS (Mailing) No. Street City State

8. COOPERATING AGENCIES: Georgia State Department of Health

9. DATE (a) Contract Approved: (b) Project Started: 9/8/59 (c) Project Completed:

SECTION II

1. BACKGROUND: Objective: To provide a comprehensive resume of stroke patients treated in the State under the restorative service program, including: Evaluation of methods, nurse training in restorative services, and effectiveness of improved services to stroke patients.

2. METHODOLOGY: Collect and analyze data from this program for use in developing materials for stimulating and guiding stroke activities in other States and localities.

A second contract was issued 2/29/60 to assist the State to tabulate, analyze and prepare a report from the material gathered.

3. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

a. HDCP (Contract) Amount: 73520--$15,000.00
73762-- 20,250.00
$35,250.00

MOBILE CRUISE RECORD

PROJECT NO. M9-44

CONTRACT NO. 73149

PROJECT I

1. TITLE: Design and Evaluation of the Dynamic-Acoustic Tracker

2. TYPE Demonstration

3. SECTION Administrative

4. RESPONSIBLE

5. FEDERAL (DOD) Advisor Navy

6. CONTRACTOR Control

7. RESPONSIBLE (Field) Joseph E. Mahoney, M. D.

8. ADDRESS Ave. (Street)

(Mailing) City State

9. DATE (a) Contract (b) Project (c) Project
Approved: Started: 6/15/5 (Engineering)

10. OBJECTIVE/SCOPE: Develop Simple Description of Site:

11. DESCRIPTION: (Limited) To provide a comprehensive response of sonar platforms control in the State navel dig xcal/public security program, implementation. Evaluation of existing (data) training activities in operational capability and increased security to defend military.

12. REQUIREMENT: Coiled and display sonic from this proposal Contract in channeling resources, by estimating and auditing tracks activities in other bases and facilities.

a prime contract was issued N/A/VOC to assist the state to evaluate existing and purchase a sonic from the central Military.

13. EXPLANATION PROJECTION:

m. YOU (Contract) Account: 73230-45-3/40-03

n. Identifying numbers:

PROJECT CONTROL RECORD

PROJECT NO. HD-45

SECTION I

CONTRACT NO. 75823

1. TITLE Death Rate Differential, White and Colored
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSONS (HDCP) Herbert Sauer and Milton Nichaman, M.D. (HDCP Officer (Field assignment
5. LOCATION Charleston, South Carolina
6. RESPONSIBLE PERSON (Field) Edwin Boyle, Jr., M.D., Director (Lipid Metabolism (Laboratory
7. ADDRESS (Mailing) No. 55 Street Doughty City Charleston, South Carolina State
8. COOPERATING AGENCIES: South Carolina Medical College
9. DATE (a) Contract Approved: (b) Project Started: 4/25/60 (c) Project Completed:

SECTION II

1. BACKGROUND: The contractor has access to a population group which may provide significant clues to the difference in death rate between whites and non-whites.

2. METHODOLOGY:

e. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

a. HDCP (Contract) Amount: $8,000.00

Customer Service Manual

Revision No. Page

Section 3

Contract No. 1383

Title: Wood Base Differential, Mill, and Cutter

Issued: Approved:

Author: Approved by:

Subject: Approved Supplier

Title (Revised) Revision Date Approved By

Location (Field) Revision Date Approved By

Location (Field) Revision Date Approved By

Location (Field

PROJECT CONTROL RECORD

PROJECT NO. HD-46

SECTION I

CONTRACT NO. 74516

1. TITLE Food Patterns as Compared to Pathological Information on Coronary Arteries.
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP) Marjorie Cantoni
5. LOCATION New Orleans, Louisiana
6. RESPONSIBLE PERSON (Field) Miss Margaret Moore, Nutrition Director
7. ADDRESS (Mailing) No. Street: P.O. Box 360; City: New Orleans; State: Louisiana
8. COOPERATING AGENCIES: Louisiana State Board of Health
9. DATE (a) Contract Approved: (b) Project Started: 5/31/60 (c) Project Completed:

SECTION II

1. BACKGROUND: Need for study: Although comparisons between food patterns and coronary arteries have been made in some other countries, it has not been done before in the United States.

2. METHODOLOGY: Obtain dietary information to describe food patterns of the Orleans Parish population by interviews with families of autopsy cases (ages 30-40). The dietary information will be compared with pathological information on coronary arteries.

   A selected sample will be drawn from available autopsy reports, including male and female, colored and white, and a cross section of economic and educational levels.

3. ESTIMATED DURATION: 6/30/62

4. SUPPORTING AGENCIES:

   a. HDCP (Contract) Amount: $25,000.00

PROJECT CONTRACT RECORD

PROJECT NO. MD-66

SECTION I

CONTRACT NO. 79318

4. TITLE Road Projects as Compared to Psychological Information on Opposing Attitudes.

Abstract.

2. TYPE Research

3. SUBJECT Aluminosilicoboria

4. RESPONSIBLE

PERSON (PROJ) Marjorie Cantrell

5. LOCATION New Orleans, Louisiana

6. ENTHUSIASTIC

PERSON (Field) Miss Penrose Moore, Motivation Director

7. ADDRESS No. Street

City State Zip

(Mailing)

8. CONTRACTING AGENCIES: Louisiana State Board of Health

Key Workers Institution

9. DATE (a) Contract (b) Started (c) Project

Approved: Started: 1/1/60 Completed:

SECTION II

1. ABSTRACT: Road tar study: Although complications delayed road pavings and economy materials have been used, the same objectives remained, it has not been done before in the United States.

2. DESCRIPTION: (Begin directly below in descriptive word pattern of the Oxford Movies motivation by interviews with homelisted retired cards (Code 30-40). The distance information will be combined with technological information on economic effects.

A selected sample will be drawn from available memory papers, including music and movies, colored and white, and a cross section of economic and educational levels.

3. KEY WORD DESCRIPTION: KJJ0/0S

4. BRIEF DESCRIPTION:

5. ISBN (Computer) Account: 085-009-06

PROJECT CONTROL RECORD

PROJECT NO. HD-47

SECTION I

CONTRACT NO. 74585

1. TITLE Study of Relative Effectiveness of Different Screening Tests in Diagnosing Early Heart Disease.
2. TYPE Research - Screening
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP)
5. LOCATION San Francisco, California
6. RESPONSIBLE PERSON (Field) Lester Breslow, M.D.
7. ADDRESS (Mailing) No. 2151 Street Berkeley Way; City Berkeley; State California
8. COOPERATING AGENCIES: California State Department of Health
9. DATE (a) Contract Approved: 6/22/60 (b) Project Started: (c) Project Completed:

SECTION II

1. BACKGROUND: In 1951, approximately 4,000 longshoremen in the San Francisco Bay area participated in a Health Department screening program which consisted of a number of tests designed to identify early heart disease.

2. METHODOLOGY: It is proposed to rescreen the survivors of this group to evaluate the relative effectiveness of the various test to diagnose early heart disease. Screening and follow-up records are available on IBM cards at the State Health Department. Interim information is available from the records of: Kaiser Health Plan files, Welfare files, and State Vital Records (deaths). The 1960 screening will be modeled after the 1951 project. Results will be reported on the comparison of the findings of the two programs and the morbidity and mortality experience of the group during the interim years.

3. ESTIMATED DURATION: 10/31/61

4. SUPPORTING AGENCIES:

a. HDCP (Contract) Amount: $35,222.00

1. TITLE: Study of Selective Effectiveness of Different Screening Tests in Detecting Early Uterine Cancer

2. TYPE: Research - Extension

3. SECTION: Microbiology

4. PERSONNEL:

PERSON (PROJ)

5. LOCATION:

6. PERSONNEL: Dean, Medical Sch.; Professor, Obstetrics

PERSON (Field) Clinical Professor, Gynecology

7. ADDRESS: 3171 Berkeley Way, Berkeley, California

(Mailing)

8. CONTRACTING AGENCY: California State Department of Health

9. DATE: Approved: 8/15/60 Terminated: (a) Planned: (b) Projected: Completed:

10. BACKGROUND: In 1951, approximately 4,000 Intraepithelial Carcinoma of the Cervix were discovered in a Health Department Survey. Early cancer detection is a number of these detected by

11. RESPONSIBILITY: It is proposed to determine the survivors of this Intraepithelial Carcinoma of the Cervix Cases to Ultimate Outcome. Indicate Extent, Nature and Degree of the Study Design: Retrospective and Prospective Cases are available. Records will be reviewed on the Cases Detected by Cytologic Screening Tests. Results will be Compared with Those Obtained by Other Methods. Problems and the Possibility and Morality Explored. Data Will Be Analyzed After the Interim Years.

12. SUPPORT: 10/1/60

13. SUPPORT: Applied For:

14. PROG (Continued)

Amount: $52,500

Project No. 75-61

Contract No. 12305

Account 2

PROJECT CONTROL RECORD

PROJECT NO. HD-48

SECTION I

CONTRACT NO. 73820

1. TITLE Serum Cholesterol and Blood Glucose Laboratory Tests
2. TYPE Research - Demonstration
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP)
5. LOCATION Washington, D. C.
6. RESPONSIBLE PERSON (Field) Thomas Perry, M.D., Pathologist (George Washington University Hospital
7. ADDRESS (Mailing) No. 901 Street Twenty-third
   City Washington State D. C.
8. COOPERATING AGENCIES:
9. DATE (a) Contract Approved: (b) Project Started: 4/19/60 (c) Project Completed:

SECTION II

1. BACKGROUND: Objective: To determine the simplest and most accurate laboratory test for serum cholesterol, blood glucose and other determination.

   To prepare standard laboratory procedures and forms to be demonstrated by use as part of the periodic health examination as one of the scientific exhibits at the 1961 American Medical Association convention in New York City. The findings will be correlate the finding with the physician's physical examination. The Contractor will review and test various methods and provide a report of his finding to the Heart Disease Control Program.

2. METHODOLOGY:

3. ESTIMATED DURATION: 9/26/60

4. SUPPORTING AGENCIES:

   a. HDCP (Contract) Amount: $1,000.00

REQUEST GRANTOR MISSED

PROJECT NO. MH-68

SECTION I

CONTRACT NO. 13220

1. TITLE Serum Cholesterol and Blood Glucose Laboratory Tests

2. TYPE Research - Demonstration

3. DURATION Approximately

4. PERSONNEL

EXPERT (MDCs)

5. LOCATION Washington, D. C.

6. INSTITUTIONS

7. ADDRESS (Field) Thomas Publ. Co., Technological/Industrial Hospital

(Hotels) Not Yet Published

City Zone State

8. CONSULTING PERSONNEL Washington, D. C.

Name (a) Institute (b) Service (c) Project

Approved: Disapproved: Deferred: (Explain) (Completed)

SECTION II

9. DESCRIPTION: Objective: To determine six analytic and wet accurate laboratory tests for serum cholesterol, blood glucose and other determinations.

10. Justification: To provide adequate laboratory service and tests to be demonstrated by the project of the Pacific Health Action Association in Washington, D. C. in addition to the Pan American Medical Association in connection in New York City. The Clothing will be centralized and Standing with the physician's general examination. The Corporation will review and test various methods and provide a report of its finding to the Heart Disease Control Program.

11. Methodology:

12. Evaluation Criteria:

13. Budget (One year)

Amount: $1,000.00

PROJECT CONTROL RECORD

PROJECT NO. HD-49

SECTION I

CONTRACT NO. 74975

1. TITLE Determination of Nutritional Aspects of Stroke Patient Rehabilitation.
2. TYPE Demonstration
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP) (1) Marjorie Cantoni (2) Arleigh Sawyer
5. LOCATION Harrisburg, Pennsylvania
6. RESPONSIBLE PERSON (Field)
7. ADDRESS (Mailing)
8. COOPERATING AGENCIES: Pennsylvania State Department of Health
9. DATE (a) Contract Approved: (b) Project Started: 4/19/60 (c) Project Completed:

SECTION II

1. BACKGROUND: There is a gap in our knowledge of the nutritional aspects of stroke patient rehabilitation. It is believed that the patients' maintenance and rehabilitation is affected by his nutritional patterns, habits and status. There is a need for study of these factors and for the development of a workable nutritional program for the use of the private physicians and others caring for stroke patients in home environments.

2. METHODOLOGY: Information will be collected by nutritionists' interviews with patients and their families at the time of hospital evaluation and at intervals thereafter. Schedules will be developed for recording data for tabulation and analysis at the end of the project. Nutritional counseling will be available at any time requested by physician.

3. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

   a. HDCP (Contract) Amount: $5,000.00

MONITOR CONTROL RECORD

PROJECT NO. ME-49

CONTRACT NO. 3893

1. TITLE: Determination of Statistical Average of Tensile Material Properties
2. TYPE: Determination
3. DIRECTION: Investigators
4. DIVISION: Mechanical
5. BUDGET (USCG): (1) Implied Contract (2) Actual Payment
6. SUBMITTED: Materials, Engineering
7. PERSONNEL (USCG): (1) Project Engineer
8. CONTRACTING AGENCY: Engineering Experiment Station, Georgia Institute of Technology
9. DATE: (a) Contract Approved: (b) Project Started: (c) Project Completed:

ABSTRACT

Tensile material properties are of prime importance in the design of structural members. This is a report of a research project to determine the statistical variation of tensile properties of a material. Specimens of the type of the ordinary tensile test were used. Physical properties and mechanical properties were determined for each specimen. Statistical methods were used to analyze the data.

KEY WORDS: Tensile material properties, statistical analysis, mechanical properties.

Tensile material properties are of prime importance in the design of structural members. This is a report of a research project to determine the statistical variation of tensile properties of a material. Specimens of the type of the ordinary tensile test were used. Physical properties and mechanical properties were determined for each specimen. Statistical methods were used to analyze the data.

ACCESSION NO.

(a) Contract

(b) Project

Abstract:

Keywords:

Publication Date:

Price (Contract):

Price (Sales):

Pages: 38,800.00

RESEARCH PROJECT DESCRIPTION

Date: August 5, 1960

1. DIVISION: Special Health Services

2. BRANCH: Heart Disease Control Program

3. SECTION: Rheumatic and Congenital Heart Diseases

4. LOCATION OF PROJECT: Washington, D. C.

5. DIVISION PROJECT NUMBER
SHS-HD-51

DIVISION CONTRACT NUMBER
SAph 73519

6. DATE WHEN INITIATED
Sept. 25, 1959

7. PROJECT TITLE: Investigation of Rheumatic Fever Patients Becoming Ineligible for Bureau of Maternal and Child Health Services

8. INVESTIGATOR: Mr. William D. Rouse

9. PARTICIPATING ORGANIZATION: District of Columbia Department of Public Health

10. OBJECTIVE AND RESEARCH PLAN: Purpose of the investigation was to get the data on the number of persons on the Bureau of Maternal and Child Health Rheumatic Fever Registry who reached age 21 during three selected years. This by statute rendered the patients ineligible for medical services, including prophylaxis, from the Bureau. It was further intended to follow up the patients reaching age 21 during the year 1959 in order to find out who continued prophylactic regime and remained under medical supervision, to report under categories the Rheumatic Fever patients on the register since its inception; also to survey all local Health and Welfare agencies in order to determine what services were available to the adult Rheumatic Fever patient.

There were 5,771 patients on the register in the years 1948 through 1959, excepting 1949, for which figures were not available. Four reports were filed under the contract, which cited the facilities available to the adult rheumatic fever patient in the District of Columbia, as well as the results of the patient follow-up. Investigation completed June 30, 1960.

SUNDANESE PERSON'S DECLARATION

PROJECT CONTROL RECORD

PROJECT NO. HD-52

SECTION I

CONTRACT NO. 71726

1. TITLE Relationship of Dietary Fat to Coronary Artery Disease in Employees of the Western Electric Company in Chicago
2. TYPE Research
3. SECTION Atherosclerosis
4. RESPONSIBLE PERSON (HDCP)
5. LOCATION Chicago, Illinois
6. RESPONSIBLE PERSON (Field) Oglesby Paul, M.D. (Univ. of Illinois, Health Survey Project)
7. ADDRESS (Mailing) Bldg. 56-1 Room 16, Hawthorn Station, Western Electric Co. Chicago, Illinois
8. COOPERATING AGENCIES:: Presbyterian and St. Luke's Hospitals 1753 W. Congress Street, Chicago, Ill.
9. DATE (a) Contract Approved: (b) Project Started: 5/15/57 (c) Project Completed: 1/16/60

SECTION II

1. BACKGROUND: Objective: To aid in the financing of the statistical analysis of the nutritional data collected in the longitudinal study of the "Health Survey Project, Hawthorn Plant, Western Electric Company.

2. METHODOLOGY: The original contract (5/15/57) $10,000.00) was for the purpose of developing protocol to study the dietary habits of 2,000 white males more than 40 years old in Chicago to determine the nutritional aspects of a longitudinal study.

   The protocol was completed and submitted 6/20/57 and the contractor agreed to complete follow-up and make reports available over the five-year period.

3. ESTIMATED DURATION:

4. SUPPORTING AGENCIES:

   a. HDCP (Contract) Amount: FY 1957--$10,000.00
   1959-- 2,500.00
   $12,500.00

THE ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR

The National Heart Institute

Calendar Year 1960

by

Dr. Robert W. Berliner

This introduction to a review of the intramural research of the National Heart Institute will be brief. The material contained in the following pages, largely as reported by the leaders of its major research groups, speaks for the Heart Institute far more effectively than can any words of introduction. We believe the reports that follow combined with the published bibliography of the Heart Institute constitute a record of considerable achievement. That much of it is directly pertinent to the categorical responsibilities of this Institute will be apparent; we take considerable pride in the fact that this is accomplished without restriction of the freedom of the individual scientist to pursue, within the resources available, those problems which excite his intellectual curiosity. We believe the high level of scientific achievement is not separable from this freedom.

Several organizational changes have been effected in the Heart Institute. These are not reflected in the report which follows since the changes were accomplished toward the end of the year. The General Medicine and Experimental Therapeutics Branch has been for some two years an organizational designation without real operational meaning. Its three sections, Experimental Therapeutics, Clinical Endocrinology, and Cardiodynamics, have each operated quite autonomously. This independence has now been recognized and the Experimental Therapeutics and Clinical Endocrinology groups have been given branch status under the leadership of Drs. Albert Sjoerdsma and Frederic Bartter respectively. The Cardiodynamics Section has been combined with the Cardiology Section from the Surgery Branch to establish the Cardiology Branch under Dr. Eugene Braunwald. That portion of the Cardiodynamics Section which has been the responsibility of Dr. Donald Fry has been established as the Section on Clinical Biophysics within the Cardiology Branch.

LABORATORY OF CELLULAR PHYSIOLOGY AND METABOLISM

Section on Cellular Physiology

The program of the Laboratory of Cellular Physiology and Metabolism, Section on Cellular Physiology, is aimed at the understanding of what might be called the "design" of protein molecules and with the factors concerned in their structure, biosynthesis and mechanism of action. This broad problem is being attacked from a number of directions involving biochemical, genetic and physical investigations. The major premise of the research is that the biological and physical properties of proteins are predictable from a knowledge of covalent structure alone and that much of the mysticism attached to proteins has grown out of lack of understanding of proteins as organic molecules. Thus, it is felt that such phenomena as irreversible denaturation and the loss of function resulting from treatment by such agents as heat, urea, etc., do not necessarily reflect irreversible changes in structure, but rather ineptitude and lack of information on the part of investigators. The following reports of specific projects summarize the experimental details and future plans of individual segments of the program.

1) In previously reported studies it has been demonstrated that the four disulfide bridges in ribonuclease can be cleaved by reduction with mercaptoethanol and that the resulting inactive product can be converted to the original native molecule by simple exposure to atmospheric oxygen. This work has been extended in the direction of the elucidation of the factor concerned with the proper pairing of half-cystine residues. It is now clear from an examination of the effects of a variety of chemical and physical agents that the matching of proper half-cystine residues in disulfide bond formation is under the control of a large number of side chain interactions involving hydrogen bonding, electrostatic attractions and repulsions, and Van der Waal's forces. For example, when fully reduced ribonuclease, containing eight sulfhydryl (SH) groups per mole, is allowed to reoxidize in the presence of 8 M urea, all of these SH groups disappear and a tightly coiled spherical molecule is produced which is completely inactive in spite of the fact that it has physical properties much like those of native ribonuclease. Upon re-reduction of this substance followed by reoxidation under optimal conditions of pH and protein concentration, a material which is indistinguishable from the native enzyme is obtained in yields up to 95%. Such an experiment demonstrates the importance of hydrogen bonds in the process since urea is known to be a strong hydrogen bond rupturing agent. The importance of the other interactions mentioned above may be demonstrated by the use of other substances and conditions.

Studies of this sort suggest strongly that all of the information required for the formation of a completely cross-linked and internally coiled globular protein is coded into the amino acid sequence of the molecule and that no further information is necessary to direct the formation of the complicated three-dimensional structure that characterizes this particular enzyme.

The studies outlined above have involved the development of new methods for handling reduced proteins which add considerably to the simplicity and reproducibility of the work. It is planned during the coming year to apply these methods to a variety of other protein molecules in an effort to test the generality of the hypotheses mentioned above. Preliminary findings show that another enzyme, lysozyme, behaves in the same manner as ribonuclease in relation to the reversibility of its reduction and reoxidation.

2) Continuing efforts are being made to work out the structures, within biologically active proteins, that are essential for such functional processes as catalysis and hormonal stimulation. These studies involve specific degradation of ribonuclease and other proteins in an attempt to arrive at minimum structures that are still functionally competent. It has been possible to mask almost all of the positively charged side chain groups of ribonuclease by the chemical addition of strands of polyalanine to the amino groups of the lysine residues without loss in enzyme activity or of the ability to regenerate active material after preliminary reduction of disulfide bridges. It has also been possible to remove several amino acids from the carboxyl-terminal end of the enzyme without inactivation although more extensive degradation has led to complete loss of activity. The degradative and reductive experiments now make possible the preparation of fragments of the reduced form of ribonuclease which will serve as raw materials for preliminary studies on the organic synthesis of portions of the protein that might show enzymatic activity. Such work is of importance since it is necessary to show that those parts of the total structure responsible for activity, as indicated by degradative studies, are the same as those that will be required in the eventual synthesis of an active substance.

3) Since only certain parts of enzymes appear to be essential for function, it seems likely that such parts of the structures would be less likely to undergo change during speciation and evolution in general. If this hypothesis is correct, it seems likely that a comparison of the same enzyme from different species and from mutants of the same species should indicate common denominators of structure associated with function. Along these lines, a number of different lysozymes are being investigated, both structurally and with relation to the genetic "information"

of the organism in question. The structure of lysozyme from chicken egg white and from bacteriophage T4 are now under active investigation and structures of lysozymes from a variety of other species of birds are being screened in a qualitative way. During the past year a chromatographic technique has been perfected in this laboratory which permits the separation of the component peptides of egg white lysozyme following reduction and alkylation of sulfhydryl groups and trypsin digestion of the protein. This technique consists of a gradient elution, from phosphorylated cellulose ion exchange columns, using pH and salt gradients of a volatile buffer, ammonium acetate. The amino acid analyses of the peptides obtained by this method provide the background information and material necessary for further sequence work. Current attempts at specific blocking of selected sites during trypsin digestion are aimed at providing information which will make it possible to assemble the peptides in their proper order.

Among the mutants of bacteriophage T4 several have been isolated that produce lysozymes with physical properties differing from those of the enzyme in the original standard strain of phage. The lysozyme of one such mutant has been examined in detail and it has been found that its structure differs by a change in amino acid sequence from that of the standard enzyme. Similar examination of other mutants should provide information to test the hypothesis that the amino acid sequence of an enzyme is determined, in a direct fashion, by the sequence of genetic "information" coded into the DNA molecules that determine heredity. The selection for interesting enzyme mutants will be continued and the fine structure genetic map extended. Ultimately, it is by the determination of the nature of the changes produced in the enzyme as a result of mutation (particularly when induced by chemical mutagens with known mechanisms of action) that we hope to begin to gain an insight into the nature of the "genetic code."

4) Studies on the biochemistry of muscular contraction have continued with special emphasis on the chemistry of the myosin molecule. In earlier studies it was demonstrated that myosin is probably made up of three physically and chemically identical polypeptide chains, each coiled in the form of a helix, the three helices being wound together in the form of a rope. Present studies now suggest that these three strands may be completely dissociated from one another and under suitable conditions be made to reassociate to yield the original parent molecule. The results suggest that the subunit is so designed that its proper aggregation is predetermined by the location of interacting groups along the chain much in the way that such groups determine the re-formation of native ribonuclease from the fully reduced molecule as described above. The structure of the individual subunits is being studied by the preparation of enzymatic digests and the separation of the resulting peptide fragments

for subsequent determination of sequence. The alignment of these fragments into the proper order should eventually yield information on the spacing of interacting groups along the chain and may make it possible to explain the aggregation process on a rational chemical basis. In the course of these studies, a careful determination of the molecular weight of myosin by light-scattering methods has indicated, in accordance with the chemical studies, that the native molecule is made up of three subunits, each with a molecular weight of approximately 200,000. These studies have elucidated some earlier experimental discrepancies which led to the postulation of a considerably lower molecular weight for this protein.

It was observed some time ago that the ATPase activity of myosin, the protein unit of the contractile mechanism, exhibits a biphasic response to titration of the SH groups, with a 3-4 fold stimulation of ATPase activity when approximately 1/2 of the SH groups are titrated, and complete inhibition on further titration. The initial phase of this process is also characterized by a marked alteration in the substrate and activation specificities of the protein. During the course of the titration, the ability of the molecule to react with another muscle protein, actin is lost. This reaction is widely regarded as important in the mechanism of contraction. Identification of the amino acid composition of those portions of the molecule involved in these processes and their localization in the molecule should provide considerable insight into the biochemical process of muscular contraction.

Treatment of myosin with sufficient radioactive N-ethylmaleimide to react with one of its sixteen sulfhydryl groups leads to a marked acceleration of its calcium activated hydrolysis of adenosine triphosphate and a complete loss of its ability to hydrolyze inosine or guanosine triphosphate. Furthermore, the hydrolysis of adenosinetriphosphate is no longer accelerated by ethylenediamine tetra-acetate. This process is accompanied by the labeling of one of the cysteine containing peptides identified by the "fingerprinting" procedure. In addition, it appears that this peptide contains not one but two cysteine residues and the titration of this second group occurs during the inactivation phase of the biphasic response to SH titration.

5) Previous studies have indicated that the processes of protein biosynthesis in hen's oviduct appear to involve the mediation of certain lipid-amino acid complexes which are in a very rapid state of metabolic flux. The chemical nature of these complexes is being studied following their partial separation on chromatographic columns. It was recently found that specific sites which can distinguish L-valine from D-valine or L-serine exist in the lipids. Evidence that points to a covalent link between the carboxyl group of the amino acid and a lipid component was

obtained. Preliminary findings suggest the existence of a lipo-peptide thought to be involved in the protein synthetic process of the oviduct.

The lipid-peptide complexes are also being studied in E. coli in collaboration with investigators at the Carnegie Institution of Washington. Such cells also contain these materials and the results suggest that they may be associated with the ribosomes which are known to be actively involved in protein biosynthesis.

In a collaborative project with investigators at the National Cancer Institute, chromatographic studies are being performed on the ergastoplasm of rat liver cells. The ergastoplasm consists of a lipid membrane, studded with ribonucleoprotein granules approximately 150 Å in diameter. It is believed that "information" for making particular proteins is contained in the configuration of the nucleic acids of these granules. If this hypothesis is correct, one would expect the granules to be heterogeneous. The granules are being fractionated to determine if different granules lead to the synthesis of different cytoplasmic proteins.

6) Studies on the structure and metabolism of heparin and related mucopolysaccharides, the relation of heparin to lipoprotein lipase and the role of lipoprotein lipase in fat metabolism are being continued. Olive oil, corn oil, cream and cocoa butter were fed to rats with cannulated thoracic ducts. The chyle was collected and chylomicrons prepared from it. These chylomicrons were then incubated with pancreatic lipase and lipoprotein lipase. The free fatty acids liberated by enzymic hydrolysis were isolated and the molar percentage composition determined by gas-liquid chromatography and compared to that of the substrate triglyceride. The relative amounts of free fatty acid and glycerol produced during hydrolysis were also determined. The results show clearly that, whereas pancreatic lipase hydrolyzes preferentially fatty acids esterified at the α-position of a triglyceride, lipoprotein lipase hydrolyzes α- and β-esters equally well. Lipoprotein lipase does not appear to have a specificity among the long chain fatty acids, saturated or unsaturated.

Section on Metabolism

The Metabolism Section of the National Heart Institute has a wide range of current interests which center primarily on the chemistry and metabolism of two classes of compounds, lipids and proteins. The two are in many respects interrelated but the study of lipids is perhaps paramount and can be considered as part of an over-all program aimed at elucidating the causes of atherosclerotic heart disease.

1. The Metabolic Activity of Adipose Tissue. One of the major projects in the laboratory involves the study of the metabolic functions of adipose tissue and their control. This has been approached experimentally in several ways. The mechanisms through which the rate of fatty acid release is regulated and altered by hormones is of primary interest and several kinds of experiments have been carried out to explore the hypothesis that alterations in the rate of fatty acid release may result from changes in the rate of fatty acid esterification.

a. Pathways of triglyceride synthesis studied in cell free homogenates of adipose tissue. Glycerides are synthesized from fatty acyl Coenzyme A and α-glycerophosphate by a mechanism apparently like that previously described in liver. The adipose tissue, however, cannot convert glycerol to α-glycerophosphate as can some other tissues. The synthesis of α-glycerophosphate from other metabolic intermediates was studied in the homogenate system. Synthesis from phosphorylated intermediates of the glycolytic cycle was obtained when DPNH was added to the system. Neither glucose nor glycogen was an effective precursor in this system. Synthesis of triglyceride from diglyceride and acyl Co-A precursors has been observed in chicken adipose tissue, but the excessive thiolase activity of rat adipose tissue has prevented successful studies in that species. One interesting aspect of the studies of incorporation of fatty acids into glycerides is that lipolysis continues, even though net esterification of fatty acids is occurring. Characteristics of the lipase active in this in vitro system suggest that it is different from lipoprotein lipase.

b. Influence of Hormones on Adipose Tissue Metabolism. It has been shown that epinephrine, ACTH, and glucagon (which increase fatty acid release) all decrease the incorporation of C^{14}-palmitate into glycerides by adipose tissue incubated in vitro. Serotonin, which does not increase fatty acid release, does not alter C^{14}-palmitate incorporation; while glucose, which stimulates palmitate esterification, decreases fatty acid release. The effects of these hormones and of a series of compounds related to epinephrine on the conversion of C^{14}-glucose to triglyceride have also been studied in an attempt to elucidate the interrelationships between carbohydrate and lipid metabolism in adipose tissue. Some similar studies have been carried out in tissues obtained from rats with altered thyroid function, comparing effects in these tissues to those obtained in tissues from normal animals in order to determine the role of thyroid state in the responses of adipose tissue.

2. Studies of the physiology of adipose tissue and the mechanism of free fatty acid release. Fatty acids released by adipose tissue are carried through the plasma in association with serum albumin. The

equilibrium relationships between fatty acids and albumin were studied in 1957 and 1958 by Dr. DeWitt Goodman. During 1960 an attempt was made to measure with a stop-flow apparatus the kinetics of the formation of the fatty acid albumin complex. The only conclusion possible was that the reaction is complete in so short a time that this apparatus is not satisfactory for a quantitative measurement. It may be concluded that the formation and dissociation of fatty acid albumin complex requires a time that is short in relation to the time required for blood plasma to pass through a capillary in vivo. Further preliminary experiments aimed at measurements of the diffusion of fatty acids through albumin containing media were undertaken. These indicated that fatty acids ions cannot diffuse appreciably faster than can the albumin to which they are attached.

3. Studies of the biosynthesis of cholesterol. A major interest of one group in the laboratory has been the mechanism of action of MER-29. This compound has been found to affect experimental animals and man in the same way – namely, by inhibiting the reduction of the side chain of 24-dehydrocholesterol to yield cholesterol. As a result, the serum of patients treated with MER-29 contains a considerable quantity of desmosterol (24-dehydrocholesterol), which may be as much as 1/4 of the total sterol present. It also accumulates in rat tissues and presumably in man as well. Desmosterol appears to participate in many of the metabolic reactions of cholesterol and is found to be esterified at much the same rate. Whether it may give rise to bile acids and steroid hormones in which the side chain is lost is the subject of a current investigation. Desmosterol yields less color in the standard Liebermann-Burchard color reaction than does cholesterol, with the result that the application of standard cholesterol methods to MER-29 treated patients results in a falsely low estimate of serum sterol levels. Cholorimetric and chromatographic methods for the estimation of true cholesterol and desmosterol levels have been developed and publications currently in preparation or in press emphasize these data.

One of our investigators has had the privilege of working with Dr. George Popjak, both in London and during Dr. Popjak's tenure as a Visiting Scientist at NHI. Their collaborative work dealt with studies of the mechanisms of biosynthesis of some of the important intermediates in the pathway to cholesterol. It was found that both liver microsomes and a soluble enzyme were required for the cyclization of squalene. O_2 and TPNH were also essential. Studies with inhibitors indicated the essential participation of enzyme sulfhydryl groups in the reaction. The mechanism for the conversion of farnesyl pyrophosphate to squalene was also studied. The available evidence indicates that the mechanism of this condensation is asymmetric, one farnesyl pyrophosphate being converted to an intermediate which differs from that to which the other farnesyl

pyrophosphate is changed. There have been important technical difficulties with this study which have made it impossible to define the details so far but the study is being actively pursued at this time.

4. Studies of the hypoproteinemia and hyperlipemia of nephrosis. Clinical studies of nephrosis which were pursued in previous years have been curtailed since the departure of Dr. Howard Goodman from this Laboratory but experimental animal work has continued. Plasmapheresis has been utilized to simulate the renal loss of proteins. This procedure results in consistent and significant increases in serum lipids. The mechanism of this effect is under current investigation. The relation of carbohydrate feeding and infusion to serum triglyceride levels is also being scrutinized. Earlier experiments both in animals and in patients led to the finding that a high intake of carbohydrate, while decreasing the serum FFA level often led to an increase in serum triglycerides.

5. Relation of serum FFA levels to lipid deposition in tissue. In a study that was completed during 1960 and has been prepared for publication, investigators of the Metabolism Section demonstrated that the sustained elevation of FFA levels following norepinephrine infusion was correlated with an increased deposition of triglyceride in the livers of dogs. Research in other institutions has indicated that the uptake of FFA by tissues (heart and liver) is related to arterial FFA levels. These various findings add further conviction to the conclusion that FFA transport is regulated largely by the adipose tissue that controls its release into the blood rather than by tissues that utilize it.

6. Clinical studies of hypoproteinemia. In this study, carried out in collaboration with the Metabolism Section of the National Cancer Institute, patients with various types of hypoproteinemia have been admitted and studied. Two patients with analbuminemia have been encountered. Each of these was given S^{35}-labeled amino acids and studied for evidence of incorporation into serum proteins. Surprisingly, the serum albumin fraction, however carefully purified, contained some radioactivity. This result suggests that each subject makes at least a small amount of endogenous serum albumin and agrees well with the report from Bennhold's clinic that analbuminemics, who have never been given albumin, have traces of this protein in their plasma.

A much larger group of hypoproteinemic patients has been shown to be suffering from "protein losing enteropathy." This condition is demonstrated by testing with I^{131} PVP and I^{131} albumin. Clinical and biopsy studies of this group of patients have lead to the definition of a previously unrecognized disorder of the intestinal lymphatics, for which the term "intestinal lymphangectasia" is being proposed. The results of this study were presented at a meeting of the American Gastroenterological

Association and are being readied for publication. I^{131} PVP has been prepared regularly with the help of the Clinical Center Pharmacy Department in such quantity that it has been possible to provide it to investigators in other parts of the country and of the world. This program has resulted in the discovery of a large number of similar cases in other centers and to the appearance of several additional publications, primarily in foreign journals.

7. Studies on the structure of fibrinogen. An investigation into the basic structure of fibrinogen has been continued during 1960. The amino acid composition of this protein has been determined with exactness and the products of chemical and enzymatic hydrolysis are under study. The complexity of fibrinogen makes it unlikely that the complete amino acid sequence will be known soon but some important facts have emerged. Trypsin attacks fibrinogen and quickly degrades it into a few large fragments. The most important fragment has been isolated and appears to have a molecular weight of approximately 100,000. This may be equivalent to the spherical portions of the fibrinogen molecule seen in electron micrographs. The relation of fibrinogen structure to its role in the clotting mechanism is a basic interest in this work.

8. Studies of fatty acid transport. Experiments have been designed this year to eliminate certain irrelevant biological variation when the metabolism of different fatty acids is being compared. This is accomplished by introducing simultaneously such acids as C^{14}-labeled linoleic acid and H^3-labeled palmitic acid. These have been fed or injected as free fatty acids (FFA) or chylomicron triglyceride fatty acids (TGFA). The results so far have revealed some hitherto unappreciated differences in the rates of removal from the plasma of these two acids as FFA. This is detected by an early drop in the ratio of labeled linoleic to palmitic in plasma. This, in turn, is explained in part by a very rapid preferential accumulation of linoleic acid by the lecithin in liver. A greater accumulation of palmitic by the liver triglycerides is not sufficient to overcome the effect on the apparent plasma removal rates. This technique shows promise of permitting comparative measurements not otherwise available in events occurring as fast as the turnover of fatty acids.

Means of interpreting such experiments using tracer fatty acids have been improved by the programming of data obtained in animal and human experiments, such as the departure of radioactivity from the plasma or its appearance in the expired air, for the 650 computer. To this has recently been added electronic means of resolving sums of exponentials into components, a system developed in LTD-NHI. Combining these operations, it is possible with a few moments of calculation to obtain expressions for the probability of conversion of a fatty acid to carbon

dioxide or of its recycling within the plasma lipid compartment. Presently under study is the question of how useful such operations are in interpreting biological data of this rather standard type, and whether unique information can be so obtained.

9. Regulation of lipoprotein synthesis. The regulation of plasma lipid and lipoprotein levels has been studied both from the standpoint of the lipid and the protein moieties and from differing experimental approaches.

a. Measurement of cholesterol catabolism. The action of dietary fats on cholesterol metabolism was measured by using the turnover of bile acids as a sensitive indicator of cholesterol degradation. Preliminary data suggest that cholic acid turnover may be influenced by dietary fat, but final analyses are awaited for more certain interpretation. Studies of cholesterol degradation using measurement of the rate of appearance of end products of labeled cholesterol in feces have failed to demonstrate a significant correlation with the nature of the dietary fat.

b. Factors affecting rate of lipoprotein synthesis in vitro. It having been previously demonstrated in the laboratory that liver slices synthesized the protein moiety of serum lipoproteins, and that the rate of such synthesis was not influenced by factors increasing cholesterol synthesis, elaboration of other lipids in this system was studied. It has been observed that the presence of high concentrations of free fatty acids in the medium significantly increases the rate of triglyceride synthesis by the liver. Concomitant effects of glucose and of epinephrine were consistent with the overall hypothesis that increased mobilization of free fatty acids from adipose tissue to the liver could increase liver triglyceride synthesis and ultimately lead to higher levels of triglyceride and other lipids, and hence lipoproteins, in plasma.

c. The mechanism of epinephrine-induced hyperlipidemia. Epinephrine or norepinephrine were infused intravenously into anesthetized dogs. Rises in serum free fatty acids in all dogs were accompanied by large increases in liver triglyceride concentrations. Intraportal infusion of norepinephrine, which bypassed the effect of adipose tissue, did not change liver triglycerides. This, and the fact that the total content of linoleic acid in the liver rose (despite the fact that this acid cannot be synthesized by the dog) indicate that the fatty liver must have arisen because more lipid was mobilized from the adipose tissue via the FFA pathway than could be oxidized. The above effects were associated with some rise in serum cholesterol, but not triglycerides. It is possible, thus, to relate influences, such as stress, to rises in serum lipoproteins, through the mobilization of FFA. This mechanism has considerable

importance in understanding a number of currently vague influences upon plasma lipid levels.

10. Studies of defective lipid metabolism.

a. Measurement of plasma post-heparin lipolytic activity. There are presently no biochemical tests for segregating metabolic defects which may cause "essential hyperlipemia." Despite many studies of lipoprotein lipase activity, no attempt has been made to use a quantitative measurement of the activity of this enzyme in man after heparin administration as a means of such segregation. Such an enzyme assay has been developed, a set of optimal conditions determined, and rates of lipolysis established in a group of 25 normals, and these compared with subjects with hyperlipemia. The latter show great scatter, falling into three vaguely defined groups, equal to, lower, or higher than normal. More importantly, it was discovered for the first time, that the response to heparin in man varies with the previous diet. Low fat intake for 7-10 days depresses enzyme activity to about half of normal. It is also interesting that normal individuals with such depressed activity do not have hyperlipemia even though their enzyme levels, as measured in vitro, are lower than those in some patients with severe familial hyperlipemia.

b. Study of a new lipidosis. An apparently new disorder of lipid metabolism has been discovered in two siblings of a family from the Island of Tangier in Chesapeake Bay. It is manifested by tonsillar enlargement and discoloration, lymphadenopathy, and enlargement of liver and spleen. Such changes in the reticuloendothelial tissues are associated with accumulation of foam cells containing lipid. Practically all the excess lipid appears to be cholesterol esters. The pedigree has been established and many relatives examined during a trip to Tangier Island. The paternal and maternal grandfathers five generations removed include brothers and the pedigree is theoretically typical for the manifestation of the disease as the homozygous phenotype of a rare recessive gene. Whether a heritable defect in lipid metabolism is responsible remains to be proved. Studies in process to elucidate the mechanisms include tonsillar incubations with cholesterol precursors and measurement of plasma cholesterol turnover in both children. One fortuitous finding which is being further explored is the potential value of the tonsil for study of lipid synthesis in the RE system. It is a relatively available tissue, and slices can be easily prepared which readily incorporate precursors into cholesterol.

Section on Enzymes

The activities of the Section on Enzymes are directed toward the elucidation of the detailed mechanisms by which various compounds are metabolized in living organisms. Attention is directed to the metabolism of those substances which is poorly understood and which offers a unique opportunity to study biochemical phenomena of fundamental interest and general significance. Specifically, the current research includes studies on various aspects of lipid metabolism, amino acid metabolism, the metabolism of heterocyclic compounds, of onium compounds, and of one carbon compounds. It also includes studies on complex biochemical phenomena such as the regulation of cellular metabolism, enzyme induction, and cellular differentiation.

I. Lipid Metabolism

(a) Fatty acid synthesis in C. kluyveri. It was previously reported that cell-free extracts of C. kluyveri catalyze the incorporation of CO_2 into the unesterified carboxyl group of malonyl-CoA; this exchange reaction requires the presence of malonyl CoA and an acyl CoA derivative of a saturated fatty acid with 2-16 carbon atoms in addition to CO_2.

The cell-free extract of C. kluyveri has now been separated into two soluble partially purified protein fractions both of which are necessary for the exchange reaction. Although the mechanism has not been fully elucidated, evidence has been obtained suggesting that the exchange reaction involves the reversible condensation, with concomitant decarboxylation, of malonyl CoA and the acyl CoA derivative to form a β-keto thiolester derivative which is firmly bound to the enzyme:

$$COOHCH_2COSCoA + RCOSCoA + E \rightleftharpoons E \cdot RCOCH_2COSCoA + CO_2 + CoASH$$

The likelihood that this reaction represents the first step in the biosynthesis of long chain fatty acids is supported by the observation that a completely soluble enzyme complex precipitating between 65-95% saturation with ammonium sulfate catalyses the synthesis of stearate, C-16, C-20 and C-22 fatty acids from the same reactants, namely, acetyl CoA, malonyl CoA and TPNH.

(b) Fatty acid synthesis by adipose tissue. Submicrosomal particles derived from epidydimal fat pads of the rat have been found to contain an enzyme system capable of synthesizing palmitate and small amounts of stearate from acetyl CoA, malonyl CoA and TPNH. The stoichiometry of the synthesis of palmitate can be expressed as follows:

Acetyl-SCoA + 7 malonyl-SCoA + 14TPNH + $14H^+$ → palmitate + $7CO_2$ + 8CoASH + 14TPN^+.

The overall reaction proceeds most rapidly at pH 6.8 and is activated by mercaptans.

As with the bacterial enzyme preparation, the rat fat pad enzyme system was found to catalyze the interchange of CO_2 and the carboxyl group of malonyl CoA. The observation that the exchange reaction and the fatty acid synthesizing activity of the fat pad enzyme concentrated in parallel throughout a 60-fold purification provides additional support for the conclusion that the CO_2 exchange reaction is an integral part of the system required for fatty acid synthesis. A study of the specificity of the fatty acid synthesizing enzyme system for fatty acid acyl CoA derivatives indicated that odd numbered and branched chain derivatives could substitute for acetyl CoA. In such cases, odd numbered, iso, and anteiso long chain fatty acids were formed.

Incidental to this investigation has been the development of procedures for the non-enzymatic synthesis of β-keto acyl CoA derivatives and for the isolation, separation and quantitation of long chain fatty acids as their hydroxamate derivatives.

Propionate metabolism. The roles of biotin and vitamin B_{12} coenzymes in the metabolism of propionate have now been established in collaboration with P. Overath, H. Eggerer and F. Lynen of the Max-Planck Institute, Munich, Germany. In studies with enzyme preparations of Propionibacterium shermanii it was shown that biotin which is enzyme bound (E· Biotin) serves as the CO_2 acceptor in a reaction equilibrating propionyl-CoA with methylmalonyl-CoA as follows:

methylmalonyl-CoA + E· Biotin ⇌ CO_2 · E· Biotin + Propionyl-CoA.

The CO_2 · E· Biotin is presumed to be the carboxyl donor for the conversion of pyruvate to oxalacetate in this organism (Swick and Wood, 1959) and may react also with ADP and Pi to produce CO_2, ATP and regenerate E· Biotin (Ochoa, et al.). Vitamin B_{12} coenzyme was identified as a cocatalyst for the reversible isomerization of methylmalonyl-CoA to succinyl-CoA.

Investigations of the mechanism of enzymatic isomerization of methylmalonyl-CoA to succinyl-CoA have been made. Studies on this reaction have been hampered by lack of any model reaction in organic chemistry. Recently, however, the rearrangement of Δ-cinenic acid to geronic acid has been shown to involve a carboxyl-group transfer. The

further demonstration that the rearrangement was actually a transcarbonylation, mediated by elimination of carbon monoxide and its recapture by a resonance-stabilized carbonium ion, has prompted a reinvestigation of the methylmalonyl-CoA isomerase mechanism, particularly since the B_{12} coenzyme for the enzymatic reaction could serve as a carbon monoxide carrier through its cobalt moiety. With lamb kidney enzyme preparations entirely dependent on the addition of B_{12} coenzyme a search was made for formation of $C^{14}O$ from succinyl-1, 4-C^{14}-CoA, or its incorporation into the latter in the presence of methylmalonyl-CoA. The results were negative.

In other studies with partially purified isomerase from P. shermanii it was demonstrated that the isomerization of 2-C^{14}-labeled methylmalonyl-CoA yields 3-C^{14}-labeled succinyl-CoA. Thus, it is established that the isomerization involves a migration of the thiolester carbon atom to the α-methyl carbon of methylmalonyl-CoA and eliminates from consideration the idea advanced by others that the isomerization involves intermolecular transcarboxylation reactions.

II. The Metabolism of Onium Compounds

(a) The anaerobic fermentation of choline. Acetaldehyde has been identified as an intermediate in the anaerobic conversion of choline to trimethylamine, acetate and ethanol by Vibrio cholinicus. The latter two substances are derived from the dismutation of acetaldehyde which is catalyzed by alcohol and acetaldehyde dehydrogenases present in the extract. This dismutation is associated with the esterification of inorganic phosphate. The alcohol dehydrogenase was purified 65-fold and was demonstrated to have an absolute requirement for TPN. The aldehyde dehydrogenase has been purified about 20-fold; it is TPN specific and is maximally activated by addition of CoASH. These observations, and the presence in cell-free extracts of phosphotransacetylase and acetokinase, suggest that phosphate esterification associated with acetaldehyde dismutation involves the intermediary formation of acetyl-CoA and acetyl-P, followed by a transfer of the phosphoryl group from the latter compound to ADP with the formation of ATP and acetate. This phosphate esterification is not inhibited by dinitrophenol; it therefore probably accounts for the dinitrophenol insensitive portion of the total phosphate esterified during the fermentation of choline by cell-free extracts.

A new organism belonging to the genus Clostridium and capable of fermenting choline anaerobically has been isolated. Although considerable variation in the composition of the fermentation products is observed with different cultures, acetaldehyde, acetate, ethanol, trimethylamine and ammonia have been identified as major products. The formation of

ammonia is particularly significant since it must reflect a considerable metabolism of the tertiary amino group under anaerobic conditions. The fate of the methyl groups is under investigation.

(b) Metabolism of sulfonium compounds. In an effort to determine if the energy associated with the hydrolysis of the sulfonium bond (-21,000 cal.) can be used for cellular metabolism, an anaerobic organism capable of growing on dimethyl-β-propiothetin as the major source of carbon has been isolated in pure culture from soil by the enrichment culture technique. The fermentation balance for the degradation of propiothetin may be tentatively written as follows:

$$3(CH_3)_2\overset{+}{S}\text{-}CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3CH_2COOH + 3(CH_3)_2S + CO_2 + 3H^+ + CH_3COOH$$

The possibility that this fermentation involves a primary cleavage of propiothetin to dimethyl sulfide and acrylate which is followed by a secondary fermentation of the acrylate to propionate, acetate and CO_2 is indicated by the fact that cell suspensions are capable of fermenting acrylate to the latter products.

It has been established that cell-free extracts are capable of catalyzing the fermentation of propiothetin but the products have not been determined.

Incidental to this investigation has been the development of a new sensitive analytical method for the determination of dimethyl-propiothetin.

III. Metabolism of Heterocyclic Compounds

(a) Riboflavin degradation. It was previously shown that the oxidative dissimilation of riboflavin by an aerobic organism involves the intermediary formation of 1-ribityl-6, 7-dimethyl-1, 2, 3, 4-tetrahydro-2, 3-diketoquinoxaline. This compound is subsequently converted to 6, 7-dimethyl-2, 3-quinoxalinediol by an oxygen dependent cleavage of the N-ribityl bond. In order to facilitate further studies on the nature of the cleavage reaction and to establish the fate of the ribityl moiety, 1-ribityl-6, 7-dimethyl-1, 2, 3, 4-tetrahydro-2, 3-diketoquinoxaline-1'-C^{14} was prepared by a non-enzymatic synthetic procedure. The enzymatic degradation of this labeled intermediate is currently under investigation.

Plausible intermediates in the further enzymatic conversion of 6, 7-dimethyl-2, 3-quinoxalinediol to 3, 4-dimethyl-γ-pyrone-6-carboxylic acid and oxamide are 6, 7-dimethyl-2, 3, 5-quinoxalinetriol and 3, 4-

dimethyl- 4-pyrone-6-carboxylic oxamide. Attempts to synthesize the latter substances have been made, but as yet the desired compounds have not been obtained.

In order to simplify further studies on the organism responsible for riboflavin degradation, the factors present in yeast extract and peptone required for its growth have been determined. It has been established that methionine, lysine, threonine, valine, isoleucine and tyrosine are all needed for maximum growth. It was further determined that the degradation of riboflavin is an inducible metabolism in this organism and that ribose but not ribitol will serve as an energy source for growth.

(b) The biosynthesis of phenazine-1-carboxylic acid. The ability of various labeled metabolites to serve as a source of carbon for the biosynthesis of phenazine-1-carboxylic acid by washed cell-suspensions of Pseudomonas aureofaciens was investigated. The isotopic carbon from formate-C^{14}, acetate-1-C^{14}, lactate-1-C^{14}, alanine-2-C^{14}, glycerol-1, 3-C^{14}, threonine-U-C^{14}, glucose-6-C^{14}, pyruvate-1-C^{14}, serine-3-C^{14}, tyrosine-U-C^{14}, and tryptophan-3-C^{14} is readily incorporated into the phenazine pigment. On the other hand isotope from glucose-1-C^{14} was not incorporated. Efforts to demonstrate a common precursor role for some of these substances by means of the isotopic competition technique were unsuccessful. Thus, the extent of isotope incorporation with glycerol-1, 3-C^{14} was uninfluenced by pools of unlabeled serine, alanine, or glycine. Efforts to obtain mutant strains blocked in their capacity to synthesize the phenazine pigment were also unsuccessful. Inhibition of pigment formation has been observed with various aromatic compounds such as o-anisidine, aniline, o-phenylenediamine, anthranilic acid, etc.

Regulation of Biosynthetic Pathways

(a) Aspartate metabolism. Regulation of diverse biosynthetic pathways by changes in concentrations of the ultimate products is achieved by two distinct mechanisms: (1) a repression of the synthesis of enzymes involved in the metabolic pathway, and (2) specific inhibition of the activity of the first enzymatic step in the metabolic sequence. The first mechanism has been referred to as "repression" and the second is known as "feedback" control.

In collaboration with G. N. Cohen of the Pasteur Institute, Paris, France, it has been shown that in E. coli aspartokinase catalyzes the first step in a metabolic sequence leading ultimately to the formation of the three different amino acids threonine, lysine and methionine. Its control by either of the above regulatory processes therefore presents a unique situation in which excessive production of one of these amino acids

could lead to decreased aspartokinase activity and consequently to a deficiency in the production of the other two amino acids.

Evidence has been obtained showing that in E. coli a partial solution to this dilemma is obtained through the synthesis of two different aspartokinases which are independently controlled by the concentrations of L-lysine and of L-threonine. One enzyme is specifically and non-competitively inhibited by L-lysine and its formation is completely repressed when the organism is grown in the presence of 10^{-2} M L-lysine. The other aspartokinase is specifically and competitively inhibited by L-threonine but its concentration is not significantly influenced by growth in the presence of L-threonine. The biological implications of this discovery are supported by the further observation that in yeast, where the synthesis of lysine does not involve aspartate as a precursor, the lysine sensitive aspartokinase is virtually absent; here the threonine sensitive enzyme is the major if not the only enzyme present.

IV. The Metabolism of Amino Acids

(a) The conversion of phosphohomoserine to threonine. The mechanism previously proposed for this unique transformation in which alcohol isomerization is coupled with phosphate elimination has now been confirmed. When this reaction is carried out in 100% D_2O, exactly 2 solvent hydrogen atoms appear in newly formed threonine. A complete hydrogen degradation of threonine has been developed, and has shown that in all cases solvent hydrogen is incorporated only into the and positions. The small incorporation of solvent H^3 previously reported reflects addition of protons to a double bond 40 times faster than tritium ions. The study of the mechanism of this unique reaction is still at an early stage. While the coenzyme (pyridoxal-P) can be formulated as potentiating removal of phosphoric acid, shifting of double bonds, and oriented addition of water, neither it nor phosphate can as yet be invoked to account for the different products in the reactions: homoserine -ketobutyrate, phosphohomoserine threonine.

(b) The mechanism of sulfur transfer between homocysteine and cysteine. In an effort to elucidate the detailed mechanism whereby the reversible transfer of sulfur from homocysteine to cysteine occurs in intact microorganisms, and in order to explain the non-reversibility of this process in mammals, the metabolism of cystathionine has been investigated at the enzyme level. An enzyme catalyzing the cleavage of cystathionine to -ketobutyrate, cysteine and ammonia has been purified 800-fold from extracts of a Neurospora mutant believed to lack the ability to form cysteine and homocysteine. More slowly this enzyme catalyzes the conversion of homocysteine to -ketobutyrate and ammonia and of

cysteine to pyruvate and ammonia. Qualitative differences between it and the homologous crystalline enzyme from liver are already apparent.

(c) γ-aminobutyric acid metabolism. It was previously established that extracts of Clostridium aminobutyricum catalyze the conversion of γ-aminobutyrate to γ-hydroxybutyrate and NH_3. This conversion involves the intermediary formation of succinic semialdehyde by a mechanism which is obligately linked with transamination to α-ketoglutarate (needed in catalytic amounts) followed by DPN-dependent oxidative deamination of the glutamate formed. This is coupled with the reduction of succinic semialdehyde to γ-hydroxybutyrate. The further metabolism of γ-hydroxybutyrate to acetate and butyrate by dialyzed extracts requires orthophosphate, DPN, TPN and acetyl CoA: presumably, the acyl CoA derivatives of vinyl-acetate, crotonate, β-hydroxybutyrate and acetoacetate are involved as intermediates.

Elsden and his associates have shown that 10 μg of dry bacteria are produced for each μmole of ATP formed during the anaerobic fermentation of various substances by organisms of various kinds. In the present study it has been found that the fermentation of γ-hydroxybutyrate and of γ-aminobutyrate by C. aminobutyricum leads to the production of 8 to 9 μg of bacteria per μmole of substrate. It is therefore inferred that one ATP per mole is produced during these fermentations. This is greater by a factor of two than is anticipated from the established route of butyric acid-acetic acid fermentation and suggests that additional phosphate esterification may be available from anaerobic electron transfer reactions.

(d) Reduction deamination of glycine. It has been found that exhaustive dialysis of partially purified enzyme preparations of C. sticklandii results in the loss of ability to catalyze the reduction of glycine to acetate and the concomitant synthesis of ATP. The activity of such preparations can be restored by the addition of a heat stable factor obtained from the partially purified enzyme. The properties of this material are under investigation.

V. One Carbon Metabolism

(a) Reduction of CO_2 to methane. In studies with dried cells of Methanobacterium omelianski which catalyze the reduction of carbon dioxide to methane, it could be demonstrated, in collaboration with A. Lezius and F. Lynen, Max-Planck Institute, Munich, Germany, that carbon monoxide, formaldehyde, formate or methanol are not intermediates. CO_2 reduction in the presence of molecular hydrogen follows a long lag period during which time an extremely oxygen sensitive compound is produced. Both CO_2 and H_2 are required for the formation of this

substance which, after reaching an optimal concentration, allows methane formation to proceed at a linear rate for hours or until the substrates are exhausted. This lag period is not associated with a deficiency in the hydrogenase system nor is it overcome by the addition of ATP or ATP-generating systems. Once the oxygen labile compound is formed, it can be kept for hours in an atmosphere of pure H_2.

The overall reduction of CO_2 to methane is inhibited by a number of metabolic poisons, notably by arsenate, iodoacetate, arsenite and low levels (10^{-4} to 10^{-5}M) 2, 4-dinitrophenol. Inhibition by the latter compound suggests that coupled electron transport phosphorylation occurs during CO_2 reduction to methane. The fixation of $C^{14}O_2$ into the carboxyl groups of pyruvate and alanine by cell-free enzyme preparations was found also to be inhibited by dinitrophenol.

Coenzyme and vitamin analyses revealed that two different strains of methane producing bacteria contain exceptionally high concentrations of biotin and vitamin B_{12} coenzyme. The extraordinarily high level of these vitamins in organisms whose energy metabolism is restricted to the reduction of CO_2 to methane, is inferential evidence that they are catalysts in this biological process.

VI. The Role of Lipoate in the Metabolism of Lactate by Butyribacterium rettgeri

Previous studies by Barker and coworkers have shown that in Butyribacterium rettgeri lipoate is required for the fermentation of lactate but not of pyruvate. It is thus suggested that in this organism lipoate is required for a process other than in the oxidation of an α-keto acid. The conclusion is supported by results of the present study showing that lipoate is apparently involved in the conversion of lactate to pyruvate but not in the subsequent dissimilation of pyruvate. The oxidation of lactate to acetate and CO_2 by washed cell suspensions of B. rettgeri is obligately coupled with the stoichiometric reduction of oxidized lipoate to the reduced form. On the other hand, lipoate cannot serve as an electron acceptor for the oxidation of pyruvate.

It has been further established that the fermentation of pyruvate by cells grown on lactate (in the presence of lipoate) are quite different. With glucose adapted cells, pyruvate is dismutated to a mixture of lactate, acetate and CO_2 whereas with lactate adapted cells, pyruvate is fermented to butyrate, acetate and CO_2.

VII. The Mechanism of Pyruvic Kinase Action

A continuing problem is represented by studies of the pyruvic kinase mechanism, particularly from the point of view of reconciling the abilities of the enzyme to phosphorylate alternatively an enolic oxygen, or inorganic fluoride in the presence of bicarbonate. Perhaps the narrowest way in which these dissimilar reactions could be reconciled would be to place F^- on the enzyme surface in place of $C{=}C{-}O^-$, and $CO_3^=$ in place of $-CO_2^-$, and further to propose an intermediary acyl phosphate in both reactions. Accordingly experiments were carried out to test this hypothesis, by incubating either pyruvate or phosphoenolypyruvate with crystalline pyruvic kinase in $H^3{}_2O$, in absence of nucleotides. The enzyme was found to catalyze a slow exchange of solvent hydrogen into pyruvate, but none into phosphoenolpyruvate. This result constitutes evidence against the hypothesis.

VIII. Biochemistry of the Differentiating Slime Mold

(a) Carbohydrate metabolism. Apparent changes in the accumulation of glucose-6-phosphate dehydrogenase during differentiation of the slime mold Dictyostelium discoideum prompted an investigation, in collaboration with B. Bloom of NIAMD, to ascertain if the routes of carbohydrate metabolism undergo significant changes also. The relative yields of $C^{14}O_2$ from 6-C^{14}-glucose and 1-C^{14}-glucose were determined when these substances were incubated with the slime mold at 5 different stages of development. The ratio of CO_2 derived from the 6 carbon and the 1 carbon atom was 0.7 in the amoebae, 1.3 at pseudoplasmodium and preculmination stages and 0.3 at the fruit stages, thus indicating marked changes in the relative contribution of the hexose monophosphate-pentose-phosphate pathway and the glycolytic pathway at different stages of development. Three possible pathways to account for the ratio of 1.3 observed at preculmination were dismissed as unlikely because of the inability to detect the required enzymatic steps in cell-free extracts.

(b) Stability of isocitrate and glucose-6-p-dehydrogenases. A heat-labile proteolytic-type agent(s) present only in extracts prepared at the amoebae stage of development was discovered to specifically destroy isocitric- and G6P-dehydrogenases. These enzymes could be fully protected by the presence of their substrates. Glutamic acid dehydrogenase and glutamic-pyruvic transaminase were not attacked. Knowledge of this differential enzyme destruction as a function of the stage of differentiation has, in conjunction with related studies, suggested the possibility that enzyme activities in vivo may be controlled by sequential endogenous substrate utilization (and inhibition), thus accounting for certain metabolic changes occurring during differentiation.

IX. Enzyme Induction

(a) β-galactosidase synthesis. The accumulation of a specific enzyme upon induction could be due in whole or in part to a decreased rate of destruction, as well as to an increased rate of synthesis. In other words, a "basal level" inducible enzyme may be turning over more rapidly than other cellular proteins, and the inducer may act by combining with this enzyme and stabilizing it, thus allowing its accumulation. In an effort to detect possible differences in the turnover of inducible enzymes under conditions of high and low rates of synthesis, a 30 second pulse of highly tritiated leucine was given to exponentially growing E. coli cells in the presence, (a) of melibiose, a powerful inducer of β-galactosidase, and (b) of galactose, a poor inducer. The β-galactosidase formed in the presence of melibiose was 1/3 as radioactive as the average cellular protein whereas β-galactosidase formed in the presence of galactose was twice as radioactive as the average cellular protein. These results would be expected if increased enzyme accumulation on induction was in part due to a decreased turnover of β-galactosidase.

LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS

The work of the Laboratory may be summarized in three areas. These are 1) the development and application of new methodology for the study of lipids and related substances, 2) investigations of the occurrence, structure and properties of plant alkaloids, and 3) studies of components of the kallikrein-kallidinogen-kallidin system, and of the chemistry of human polypeptide vasodilators.

1) Lipid Methodology

Investigations of lipid metabolic problems important to atherosclerosis studies have been hampered for some time by the poor state of methodology in the field. Work on all aspects of lipids, from basic chemistry to nutrition, advanced very slowly in the past because of this situation. Some time ago this laboratory started work on the development of new laboratory techniques for studying naturally-occurring lipids and related substances, with a view to developing new methodology. Significant basic discoveries in gas chromatography had been made earlier in England, and these were taken as the starting point. During 1959 methods were developed for the study of fatty acids and related long-chain compounds including alcohols, aldehydes and bases of the sphingosine type. This work required the development of new column materials for gas chromatographic work, and these column packings are now in rather general use in this country and abroad in both biological research work and manufacturing control laboratories.

The success of these methods in providing a new and important tool for studying long-chain compounds obscured the fact that gas chromatography was in fact more than a specialized tool or a new body of techniques of limited use. Early experiments suggested that steroids and other large molecules were not amenable to separation in this way but these experiments did not distinguish sufficiently between theoretical considerations and problems of technology. During 1960 the problem of extending gas chromatographic methods to the separation of steroids and other complex compounds of natural origin was undertaken. A series of studies was made with thermostable phases not previously investigated. The requirements for supports, for detection systems, for chromatographic columns and for vaporizing systems were investigated. Previous studies with long-chain compounds provided the necessary background of experience. It was found that with new techniques many classes of high molecular weight compounds could be separated. Steroids, alkaloids, vitamins A, D, E and K, many drugs and drug metabolites, and many metabolic products of varied structure can now be separated and studied by gas chromatography. The sensitivity of these procedures, the superb

resolving power, and the ability to use complex mixtures of a few micrograms or less in total size (the ultimate sensitivity of current detection systems has never been used) are now well recognized.

2) Alkaloid Work

Studies of alkaloids of the Amaryllidaceae have been continued. Several biosynthetic studies were started in collaboration with Dr. A. R. Battersby. The biosynthetic work is being carried out primarily in England, in continuation of Dr. Battersby's present studies, and the chemical degradative work will be done here. Current experiments are designed to establish the origin of the ring systems of these alkaloids.

Structural studies were also continued. The structure of buphanamine was established, and additional degradative methods for studying the alkaloids were developed.

Additional supplies of galanthamine were isolated for physiological investigations. This Amaryllis alkaloid was introduced into therapy in Russia in 1958 in the treatment of myasthenia gravis. Several synthetic compounds, derived from the alkaloid, have been prepared for comparisons of activity.

Studies of Cassia and Ormosia alkaloids resulted in the determination of structures for jamaidine and jamaicensine. These compounds are found in seeds of Ormosia jamaicensis; material of Jamaican origin was used in the work. The relationship of the two alkaloids was demonstrated by the synthesis of jamaidine from jamaicensine. The Cassia alkaloid cassine is still under study.

The fungal antibiotic pleurotin was isolated from Pleurotus griseus. Structural studies are in progress.

Gas chromatographic techniques useful for steroid work were found to be immediately applicable to studies of complex mixtures of alkaloids. A preliminary survey indicated that many groups of alkaloids with widely different ring systems and different functional groups could be separated. An intensive study of two groups was undertaken. The gas chromatographic behavior of the Amaryllis alkaloids was investigated, since many of these compounds were available from earlier studies. The separations on a non-polar phase (SE-30) were found to be excellent, and it was found that individual alkaloids of the Amaryllis group may be characterized in this way.

Morphine alkaloids were also investigated. It was found that the complex mixture derived from the plant (crude opium) could be extracted

and the composition of the extract determined by gas chromatography. A "fingerprint" of the mixture was obtained. This work was carried out with a non-polar phase (SE-30). These results suggest that gas chromatography will become a valuable tool in alkaloid work, both in isolation and structure proof studies.

3) The Kallikrein-Kallidinogen-Kallidin System

The human vasodilating substance kallikrein owes its action to the formation of the physiologically active polypeptide kallidin. The latter compound is formed when kallikrein acts on kallidinogen, a component of human plasma. Work directed to the isolation of kallikrein, kallidin and kallidinogen has been carried on as a joint study with the Laboratory of Cardiovascular Physiology.

A kallidin fraction was prepared by the action of human urinary kallikrein on human plasma kallidinogen. Two kallidins resulted when this material was purified. These compounds, kallidins I and II, are similar but not identical polypeptides, and both have vasodilator activity. Current work is directed to the preparation of an additional supply of the polypeptides. Preliminary comparisons have been made with bradykinin, and structural work on the composition of the kallidins has been started.

Studies of human plasma, pancreatic and urinary kallikrein were continued. Partially purified fractions were obtained, and additional work is projected to obtain material of greater purity. These kallikreins are not identical, but presumably each one yields the same kallidin.

Procedures for isolating human plasma kallidinogen are under development. This component of the system is needed to complete the proposed studies of kallidin formation.

Development of New Drugs

Reserpine Analogues

It is difficult to maintain a constant clinical effect with reserpine due to its cumulative effect and its instability in the gastrointestinal tract. In collaboration with Ciba Laboratories, reserpine analogues have been developed in which the unstable trimethoxy group is replaced by a methyl group. This ether exists in two epimeric forms (Su 8842 and Su 9064), both of which are stable in the gastrointestinal tract and are completely absorbed. Furthermore, they act reversibly, sedation and effects on brain 5HT lasting only about 8 hours in rabbits. In man the drugs act rapidly and the effects are easily controlled. Preliminary trials in treatment of psychoses look promising. Furthermore, since low doses of drugs cause a persistent depletion of peripheral norepinephrine (NE) without releasing brain 5-hydroxytryptamine (5HT) or evoking sedation, they will also be tested in the treatment of hypertension.

Benzoquinolizines with Reserpine-like Action

Benzoquinolizines, like tetrabenazine, act quickly, producing reserpine-like effects, yet they release only small amounts of brain 5HT. This suggests that they act directly, perhaps by mimicking 5HT in brain, rather than through release of 5HT. A number of sedative benzoquinolizines which do not release brain 5HT are under investigation to see whether they exert typical reserpine-like effects.

Imipramine Metabolite

A metabolite of imipramine, monomethyl norimipramine, has been found to be about 25 times as active as the parent drug in blocking reserpine action. This important lead may provide a highly active compound for treatment of mental depression.

Drugs for Arthritis and Gout

A few years ago, these studies were initiated when it was found that the two metabolites of phenylbutazone isolated in this laboratory were active in man. Metabolite I exerted a pronounced antirheumatic effect; Metabolite II was strongly uricosuric. Now that Geigy Pharmaceuticals Co. has overcome certain synthetic difficulties, Metabolite I, oxyphenbutazone, has been introduced in Europe (Tenderil) as a potent, less toxic,

antirheumatic agent and it will soon be introduced in the United States. Metabolite II led to development of an analogue, sulfinpyrazone (Anturan), now available as an extremely potent uricosuric agent for treatment of chronic gout.

Except for further trials with a methylsulfone analogue, which has an extraordinary half-life, this project is terminated.

Problems of Drug Administration in Long-term Therapy

Long-term therapy creates problems in clinical toxicity, not easy to foresee from usual animal toxicity experiments.

a) Biochemically Irreversible Drugs

Reserpine and monoamine oxidase (MAO) inhibitors are drugs with actions lasting long after cessation of dosage despite their rapid biotransformation. Since the drug effects are irreversible, they are usually given in small doses over a period of weeks to reach the required effect; large priming doses can produce profound toxic manifestations in susceptible individuals. This poses problems of dosage regulation since the effects of these inherently dangerous drugs are related not to plasma levels but to the extent to which they inhibit biochemical processes. Even with small doses drugs with cumulative effects can produce a delayed toxic action. For example, when Catron (JB 516) was given to dogs in 0.5 mg/kg doses daily it produced lesions in the inferior olivary nucleus or in the pyriform cortex after 100 days of treatment.

b) Effects of Drugs on Endocrines

Tranquilizing agents (reserpine, chlorpromazine) cause hypersecretion of ACTH and perhaps other pituitary hormones when given in sedative doses. If these compounds, as is likely, stimulate ACTH secretion in man, the biochemical and physiological consequences of this action after long-term treatment become important.

c) Drug Combinations

The use of drug combinations in long-term therapy has potential difficulties. A number of drugs, MAO inhibitors for example, may depress activity of the liver enzymes that inactivate other drugs. This potential hazard should always be tested especially if the other drug is a central nervous system stimulant or depressant.

One of the two drugs may induce an increased activity of the enzyme that metabolizes the other. As an example, in using the drug combination,

phenylbutazone and aminopyrine, phenylbutazone induces an increase in the metabolism of aminopyrine so that the latter drug remains in the body for a very short time. Similarly, barbiturates may speed up the metabolism of anticoagulants and throw control of the coagulation mechanism out of balance.

If both drugs act centrally on amine receptor sites, a combination of drugs can exert effects that neither one causes alone. It has been shown that reserpine given to animals pretreated with a MAO inhibitor produces profound excitation rather than sedation. Perhaps only the fact that relatively small doses of reserpine are given to man has prevented catastrophic effects with this combination of drugs. It has also been shown that animals given an MAO inhibitor followed by imipramine (both in non-toxic doses) develop bizarre symptoms and hyperpyrexia. In patients this combination of drugs has caused peculiar motor signs, circulatory collapse and hyperpyrexia. Finally, the activity of chlorpromazine appears to be markedly increased after pretreatment with a MAO inhibitor. This has also been reported in man.

Biogenic Amines

Role of Serotonin (5HT) and Norepinephrine (NE) in Brain

Using the compound α-methyl-m-tyrosine (MMT) it has been possible to show that reserpine action is associated with the effects on brain 5HT rather than on brain NE. In rats MMT reduces the NE of brain with almost no change in brain 5HT; the animals are not sedated. However, if reserpine is now given to these animals, 5HT is lowered and sedation ensues.

With the new tool, MMT, it has been possible to show that the amines, released from binding sites and stabilized by a MAO inhibitor, elicit opposite effects in brain. Thus, if a rat is given a MAO inhibitor and then reserpine, excitation occurs as amines are liberated but stabilized. In contrast, if animals are pretreated with MMT, which depletes only the NE, and then given the combination of the MAO inhibitor and reserpine, only 5HT is stabilized in a free form and the animals now show sedation. Other experiments, which involve administration of MAO inhibitors to animals pretreated with reserpine, show that free NE at low levels in brain can elicit all the effects of large doses of amphetamine.

Brain Amines in the Newborn

Levels of brain amines were related to gross behavior in rats of various ages. At birth the amine levels are very low. The levels increase

with age and are normal when behavioral patterns are developed. Newborn guinea pigs which have more fully developed behavioral patterns have a normal content of brain amines. The newborn rat does not lack ability to synthesize the amines but is unable to store them in brain.

Mechanism of Action of Imipramine (Tofranil)

Imipramine is a clinically effective antidepressant drug which does not elicit excitation in normal animals or man. It is not an MAO inhibitor nor does it alter brain amines. The pattern of its pharmacologic effects is that of a weak chlorpromazine-like compound. Thus none of present screening procedures would show up this compound as an antidepressant. This action is dramatically evident in dogs and rats treated with reserpine. The following effects of reserpine are prevented by imipramine: depression, miosis, diarrhea, active closure of eyelids, hypothermia, potentiation of alcohol and barbiturate anesthesia, increased activity of central parasympathetic system. The activity of chlorpromazine is not affected by the drug. Preliminary data suggest that imipramine might act centrally by blocking the effects of free 5HT, but much more evidence for this is needed.

The latency in action of imipramine in animals and man has led to tests of one of its metabolites, monomethyl-norimipramine. This substance is about 25 times more active in blocking reserpine action than the parent compound. A dog given reserpine in sufficient dosage to produce coma and death is not depressed and lives if pretreated with 1 mg/kg of metabolite.

The discovery of the antidepressant effects of imipramine suggests that psychotherapeutic drugs with new kinds of activity might be discovered by screening them for the modification of the disturbances produced by other drugs.

Monoamine Oxidase Inhibitors

It had been previously shown that the antidepressant action of MAO inhibitors in animals was associated with the rise in brain NE and not in brain 5HT. It has now been established that this is also true for the new non-hydrazine inhibitors. As a result of this work, a rise in brain NE is regarded as a sine qua non for evidence that a compound is a MAO inhibitor antidepressant.

In studies of mechanism by which the inhibitors lower blood pressure, measurement of the postganglionic electrical responses induced by preganglionic stimulation of sympathetic ganglia indicates that the hypotensive effects are not due to inhibition of synaptic transmission.

The Hypotensive Drugs, Guanethidine and Bretylium

Guanethidine has been shown to lower blood pressure by depleting NE from peripheral nerve endings. Its action is unusually specific for it does not release peripheral 5HT nor either amines from brain. Bretylium, a quaternary ammonium compound, lowers blood pressure by preventing the physiological release of NE from nerve endings.

Preliminary experiments on the mechanism of action of these compounds were based on the concept suggested by Burns and Rand that postganglionic adrenergic fibers may actually be cholinergic, with acetylcholine at nerve endings liberating NE, which in turn acts on muscle as a local hormone. Bretylium (at higher doses a ganglionic blocker) would then be a specialized cholinergic blocking agent with an affinity for sympathetic nerve endings and guanethidine (at higher doses a ganglionic stimulant) a specialized cholinergie stimulant which depletes NE by releasing acetylcholine at sympathetic nerve endings. Preliminary experiments favor this view since pretreatment of rats with bretylium antagonizes the NE-releasing action of guanethidine. These drugs should prove useful tools in studies of the acetylcholine-NE relationship throughout the sympathetic nervous system.

Role of NE in Sympathetic Synaptic Transmission

The function of the brain amines is difficult to study since functional units in the central nervous system are difficult to isolate. Since sympathetic ganglia contain considerable NE, the effects of depleting the amine were studied. After ganglionic NE had been depleted with reserpine, the postganglionic response to preganglionic electrical stimulation is greatly exaggerated. Similar results are obtained by the administration of adrenergic blocking agents and by bretylium. In contrast, if free NE is pooled in ganglia by the administration of an MAO inhibitor followed by reserpine, virtually complete ganglionic blockade results until the free amine diffuses away. These results suggest that transmission across sympathetic synapses is regulated both by acetylcholine and NE and that during cholinergic ganglionic stimulation signals are put out to release NE, which in turn inhibits the effect of acetylcholine on synaptic transmission. This feedback mechanism would serve to prevent wide swings in ganglionic activity which otherwise might occur with changes in sympathetic output. Current investigations are aimed at determining whether NE and 5HT in brain also act by modulating synaptic transmission.

Mechanism of Reserpine Action

It is important to know the precise pharmacology of reserpine since it may serve to map out a neural organization in brain, modulated

by 5HT. It has been proposed that such a neuronal system integrates parasympathetic with extrapyramidal and emotional functions (trophotropic system of Hess) and is stimulated by reserpine, whereas an opposing adrenergic system (ergotropic system of Hess) is inhibited by chlorpromazine. Pharmacologically, reserpine acts oppositely to chlorpromazine in that it stimulates central parasympathetic activity; chlorpromazine in contrast depresses central sympathetic activity. These stimulatory effects of reserpine are blocked by chloralose; those of chlorpromazine are not.

Electrophysiological measurements also show the difference between two drugs. Chlorpromazine has its action on the reticular activating system by blocking stimulation from collateral input. Reserpine in therapeutic doses has its main action on the limbic system, producing a spontaneous electrical activity in the amygdala which spreads to the rest of the limbic system, but not to the neocortex. Chlorpromazine does not exert this action in therapeutic dosage.

Studies on Histamine

Although for many years histamine has been recognized as a normally occurring agent with marked pharmacologic effects, the question of its physiologic role remains unanswered. Projects in this laboratory designed to shed new light on this question have led to a sensitive and specific fluorometric method for the assay of histamine and for the enzymes responsible for its metabolism, diamine oxidase and imidazol-N-methyl transferase. It has been established that "histaminase" and diamine oxidase are the same enzyme, but that the diamine oxidase from various organs exhibits measurable differences. Experiments on the release of histamine from rabbit platelets have led to the demonstration that thrombin is an extremely potent histamine releasing agent, and that thrombin generation may be involved in the physiologic release of histamine from platelets.

Studies in Biochemical Behavior

Studies have been instituted to determine how body biochemical reactions are adapted to changes in environment through central nervous activity, and how these control mechanisms are affected by drugs through hypothalamic-pituitary-endocrine function and the autonomic system.

1) In rats, cold, alcohol and depot ACTH elicit the same rise in plasma corticosterone, plasma free fatty acids, and liver tryptophane-peroxidase indicating that cold and alcohol release ACTH. Depot ACTH, however, produces a much greater rise in liver triglycerides, suggesting that this hormone can also exert an effect on lipid metabolism not mediated

through the adrenals.

2) Reserpine and allied compounds produce hypersecretion of ACTH. They act only in doses that deplete brain 5HT by 50%, a dose also required to evoke sedation. The response is not a "non-specific stress" since isoreserpine, which is non-sedative, does not stimulate the pituitary even in lethal doses. Reserpine in doses of 2 mg/kg depletes pituitary ACTH by 80% over a period of 20 hours. Thus published reports that reserpine can prevent responses to stress are explained by the depleted pituitary. This was shown by experiments in which the pituitary ACTH of animals, cold-exposed for 20 hours, was also found to be drastically lowered, and the animals could not respond to a further stress.

3) Chlorpromazine and other phenothiazines, but only in sedative doses, also cause the pituitary to release ACTH. This is not a "non-specific" stress since non-sedative phenothiazines do not stimulate the pituitary even after huge doses. Central stimulants like amphetamine stimulate the pituitary only transiently. Thus the tranquilizing drugs produce an effect on the pituitary usually associated with stress.

4) Studies suggest that the autonomic nervous system as well as the pituitary is involved in drug-induced deposition of triglycerides in liver. Thus ganglionic blocking agents prevent both the alcohol-induced triglyceride deposition in liver and the mobilization of fatty acids from adipose tissue without affecting ACTH hypersecretion.

5) More than one mechanism is involved in aberrations of lipid metabolism induced by drugs. Carbon tetrachloride, but not alcohol, for example, produces the deposition of triglycerides in the liver of hypophysectomized rats. Drug induced changes in lipid metabolism are effected by at least three mechanisms: a) increased mobilization of free fatty acids from adipose tissue (alcohol, CCl_4 and ethionine), b) activation of triglyceride synthesis in liver (alcohol), and c) blocking of triglyceride removal from liver (CCl_4).

Passage of Substances Across Membranes

Membranes Within the CNS

It has been shown that if various sugars such as inulin, sucrose and mannitol are injected into the lateral ventricle they enter the brain through the ependymal lining slightly if at all. However, within 1 hour they have passed completely into the cisterna magna. Although these substances cannot enter the cerebrospinal fluid from plasma, they do pass from CSF into plasma rapidly and all at the same rate. These

results indicate that they leave by fluid flow through large pores, probably in the arachnoid villi. It is possible that the rate at which the large molecules leave is related to rate of CSF turnover.

Penetration of Drugs into Cells

As reported previously, basic organic compounds cross the red cell at rates related to lipid-solubility. Organic acids also penetrate the red cell at rates roughly related to lipid solubility, but in addition completely ionized compounds like phenol red readily penetrate. Organic anions probably enter red cells by diffusing through large positively charged pores, the mechanism perhaps resembling that involved in the entry of chloride.

Active Transport Mechanisms

Data obtained in studies of the passage of pyrimidines across everted intestinal sacs (rat) suggest that there must be an OH group in the 6-position of the pyrimidine or purine ring for a compound to interact with the pyrimidine transport mechanism, either as participant or inhibitor. The nature of substituents in the 2 or 5 position seems unimportant. Two anti-tumor agents, 5-fluorouracil and 5-bromouracil, are actively transported across the intestinal epithelium by the pyrimidine mechanism. These are the first examples of drugs that are absorbed by active transport. Although purines (xanthine, uric acid, etc.) block pyrimidine (uracil) transport, they are not themselves transported.

The transport of pyrimidines is an adaptive process under hormonal control. Starvation of rats, for 4 days, increases the active transport of uracil by about 600% without the changing passive component. Hypophysectomy, but not adrenalectomy, also increases active transport by 250%. It is possible that lowered food intake is the common factor in starvation and hypophysectomy.

Transport of Catecholamines by Brain and Other Tissues

The previously reported finding that 5HT is taken up in platelets by active transport has led to experiments designed to ascertain whether neurohormones in general and catecholamines in particular are also held in neurons by a "pump" mechanism. An important finding has been that labelled NE is also taken up by tissue slices by a mechanism which obeys all the criteria of active transport. This process is blocked by cardiac glycosides and by reserpine. The process is present in brain, heart, kidney and pineal gland and absent from liver and muscle. Calculation of uptake of labeled amine indicates that the endogenous NE is present in

tissue in two pools, only one of which is readily miscible with exogenous amine. This fits a picture of "free" amine in neurons maintained by the pump mechanism, and amine in granules bound to some cellular constituent.

A number of drugs inhibit uptake of NE in vitro, perhaps by competing for the transport process. These consist of sympathomimetic (cocaine and amphetamine) and sympatholytic (chlorpromazine) agents, depleters of tissue catecholamines (guanethidine) and thyroxine. Most of these compounds are known to enhance the action of administered catecholamines. This suggests that the uptake mechanism is an important means of terminating biological action of catecholamines.

Effect of Ouabain on the Turnover of Phosphatidic Acid, a Proposed Sodium Carrier

The synthesis of phosphatidic acid in rabbit brain slices is inhibited by ouabain in a concentration as low as 10^{-7} M and is 50% inhibited at 10^{-5} to 10^{-6} M. Higher concentrations of ouabain do not inhibit the synthesis of phosphatidic acid any further. This suggests that the steroid is inhibiting the synthesis of only certain components of a more complex mixture.

Drug Metabolism

1) Substances (antimetabolites) metabolized by enzymes of intermediary metabolism.

6-Chloropurine (6-ClP) and its metabolite, 6-chlorouric acid, markedly inhibit the xanthine-oxidase uricase pathway. Both xanthine oxidase and uricase are blocked in vitro at concentrations corresponding to tissue levels in vivo. 6-ClP inhibits synthesis of RNA and DNA only slightly but markedly inhibits total lipid synthesis in liver slices.

6-Methylaminopyrine (methylated adenine or MAP) is a purine of unknown function occurring in trace amounts in cytoplasmic RNA. A methylating enzyme in rat liver homogenate transfers a methyl group from labeled methionine to form MAP mainly in cytoplasm.

2) Substances acted on by non-specific enzymes not involved in intermediary metabolism.

a) Mechanism of Microsomal Drug Oxidation. This mechanism continues to command attention since it involves direct utilization of oxygen from air; it may share the same active oxygen donor as cholesterol; and a similar mechanism may hydroxylate steroids to form corticoids.

In the last report it was proposed that TPNH + O_2 react with a constituent of microsomes to form a hydroxyl-donor which oxidizes substrate, and in absence of substrate forms H_2O_2. However, kinetic studies now show that the H_2O2 cannot be formed from the hypothetical hydroxyl donor.

In view of the fact that cholesterol is formed in microsomes by a TPNH-requiring enzyme it may be of some importance with respect to mechanism that triparanol (MER 29) used clinically to block cholesterol synthesis also inhibits the metabolism of many drugs in vitro.

b) Induced Enzyme Formation. The mechanism by which many drugs increase the ability of rats to metabolize the same or closely related drugs is being actively pursued. The increased enzyme activity produced by pretreatment of rats with phenobarbital, phenylbutazone, chlorcylizine (an anti-histaminic) and aminopyrine, is paralleled by accelerated drug metabolism in the intact animal and by a shorter duration of drug action. In man phenylbutazone also accelerates the metabolism of aminopyrine, and barbiturates seem to enhance the metabolism of certain anticoagulants. In rats the inductive effects are not prevented by starvation, by adrenergic blocking agents, or by lowered thyroid activity.

Studies with Ascorbic Acid

Many drugs not only induce the activity of drug enzymes in liver microsomes but stimulate synthesis of L-ascorbic acid by mechanisms that do not involve the pituitary-adrenal system. In studies of this mechanism of increased ascorbic acid synthesis it was found that UDPG dehydrogenase, a key enzyme in the series of reactions:

glucose ⟶ uridinediphosphoglucose ⟶ (UDPG dehydrogenase) uridinediphosphoglucuronic acid ⟶ D-glucuronic acid-1-PO_4 ⟶ D-glucuronic acid ⟶ L-gulonic ⟶ L-ascorbic

is stimulated by methylcholanthrene. In addition, this hydrocarbon increased activity of enzymes which metabolize ascorbic acid. The possibility has been mentioned in other reports that ascorbic acid may have a role in drug metabolism. In accord with this view the muscle relaxant action of Flexin is unusually prolonged in ascorbic deficient guinea pigs due to decreased activity of the Flexin-metabolizing enzyme in liver microsomes. However, other factors such as lowered protein intake might also explain this effect.

Of unusual biochemical interest is the finding that the 5-carbon analogue of ascorbic acid, L-erythroascorbate, can be formed from ascorbic acid in rat liver (decarboxylation of ascorbic to L-xylonic acid, followed by conversion to the ascorbic analog). The role and function of this substance might be of considerable interest.

Some of the symptoms of scurvy in guinea pigs are reversed by 3-methylcholanthrene by an unknown mechanism. These include a marked reduction in incidence of hemorrhage, reduction of bone resorption, preservation of dentinogenesis and prevention of death of odontoblasts.

Chemical Inhibition of Cholesterol Synthesis

Because of the possibility that compounds which inhibit conversion of mevalonic acid to cholesterol might not affect other metabolic processes, the search for antagonists has continued. Three analogues of mevalonic acid, which suppress the incorporation of mevalonic-2-C^{14} into cholesterol in rat liver homogenates, have the following order of potency: 3-hydroxy-3-methylvaleric acid, Δ^{2}-3-methylpentenoic acid, Δ^{3}-3-methylpentonoic acid. These antagonists are not particularly potent.

Development of Methods of Analysis

1) A method for estimation of imipramine and its metabolite monomethyl-norimipramine has been developed. A method which separates these two substances is important since the action of imipramine is probably mediated through the highly potent metabolite.

2) A method for chlorpromazine which involves oxidation to the highly fluorescent sulfoxide. This should be applicable to humans in therapeutic dosage and may answer some of the vexing problems with this drug.

3) A method for direct determination of triglycerides in liver.

4) A new and much more rapid method for determination of ascorbic acid in tissues.

5) A method has been developed for measurement of cotinine in tissues. Since this is a metabolite of nicotine, and is fairly stable in animals, it might provide a good measure of the amount of nicotine inhaled from cigarettes.

6) A method for the tissue estimation of Librium, a widely used tranquilizer, has been developed.

The further development and application of gas chromatographic methods to problems of biochemical and medical research are continuing. Additional information has been acquired on the range and dependability of combined mass and radioactivity detection systems. This has been applied to the solution of specific problems in collaborative research projects with scientists from other laboratories and institutes.

Efforts have been continued to develop the techniques for measuring the quantity and radioactivity of labeled materials utilizing the anthracene column scintillation method previously devised in this laboratory combined with various mass detectors. Its utility in the study of sterols, a situation in which high temperatures reduced the efficiency of the original method, has been extended; the modified method provides a modified collector that can be cooled by drawing through it cool air in addition to the column effluent.

Leakage of radioactivity into the gas stream and penetrating radiation were shown to make the argon ionization detector unsuitable for low level radioactivity assay. A non-radioactive, non-destructive, high-sensitivity detector was required. Special electronic circuits were developed for this purpose.

Application of gas chromatographic methods to a wide variety of biological problems has shown that no single detector is universally satisfactory. Requirements for sample recovery or for trapping radioactivity in an anthracene column preclude the use of highly destructive electric discharges or radioactive ion sources. Radioactivity detection on anthracene columns must be checked for the presence of quenching materials in the effluent. Some ionization systems require a very low level of organic vapors to operate efficiently and in the case of fluorinated esters of the amino acids, the halogen quenched the ionization so that the RF detector proved more suitable.

These applications have indicated that the most effective use of gas chromatography requires attention to a great many details of physical conditions, as well as consideration of chemical properties. A particular example of simplification by control of conditions was the idea of adding ammonia to the carrier gas to make possible the chromatography of the amino acid esters without N-substitution.

In the course of exploring methods for using an ion chamber as a radioactivity detector, it was noted that unlabeled organic vapor flowing

through an unpolarized ion chamber produced currents the sign and magnitude of which were different for different vapors and easily measured by the vibrating reed electrometer. This may be a serious obstacle to application of the ion chamber, but the effect may be applicable as another type of detector for gas chromatography or other applications.

The study of detectors has been extended to the examination of the applicability of the hydrogen flame, as well as other detectors, to liquid-liquid chromatography effluents. Preliminary experiments have shown that it is possible to volatilize the solvent and convey the less volatile solute into the hydrogen flame to utilize the high sensitivity of this device for indicating the presence and quantity of a component coming off a column.

The collaborative research projects carried on with other scientists have made possible the analysis of all but a few amino acids, tryptophane being especially resistant and with arginine and tyrosine yielding multiple derivatives.

A study on the relationship of the composition of serum lipoproteins to the lipids of the dietary intake has been carried out in collaboration with Dr. Kayden at New York University. The results indicate that lipoprotein composition reflects the dietary fat composition for at least a day or two, and indicate the need for rigid dietary control in studies of the circulating lipids.

Several other collaborative projects were carried on as joint efforts and members of this laboratory have participated in the design of the experiments as well as in the adaptation of gas chromatographic methods to the specific problems.

The ultrasonic velocity gas chromatography detector developed in this laboratory has been improved by redesign of the cell. Its performance has been shown to conform with theory. It is unique in that, without destroying the sample, it provides high sensitivity, and very rapid, predictable response.

The application of the electronic frequency response correcting circuit for use with intravascular catheters has been improved by simplification of the procedure by circuit stabilization and development of a square wave test procedure for matching the circuit to the specific catheter.

Analogue devices for analysis of biological problems have been set up in response to specific problems presented by scientists from other

laboratories. A method of simulating regurgitation curves partially accomplished last year is now complete. Fourier series, sums of exponential functions and distribution curves are being analyzed with equipment developed in this laboratory.

The development of a general theory of transport phenomena in linear biological systems has been continued and applied to the analysis of experimental data on fatty acid metabolism. The theory is being developed to provide a formal language and method for analysis of a large number of biological problems.

The catheter tip ascorbic acid detector has been perfected. The method now has practical utility in observation of the form of indicator dilution curves for the detection of cardiac shunts and determination of the extent of heart valve regurgitation. Special waveforms, electrode designs and techniques of application are under continued study to eliminate remaining artifacts which stand in the way of quantitative determinations of flow by indicator dilution curves.

In a cooperative project with the Surgery Branch, a study was undertaken to determine the physico-chemical properties of plastics responsible for initiation of clotting or unfavorable tissue response. The contract services of Battelle Memorial Institute were engaged to provide pure specimens for in vivo testing at NIH; physical-chemical characteristics were determined at Battelle. While there were several encouraging materials in the group tested, no clear pattern of properties has yet emerged. This project was an exploratory program to initiate the application of industrial research technology, through contract, to a pressing biological problem. A university group which has shown considerable interest and competence in the field was encouraged to continue the work.

A practical method for the rapid analysis of micro-milliliter samples of renal tubular fluid is still being pursued. Sample handling and alkali metal recoveries continue to be inadequate. Sensitivity and the possibility of a relatively simple method encourage continued effort to utilize the helium glow discharge to produce the characteristic alkali metal emission.

Phosphorescence excitation, emission spectra, and emission lifetimes have been determined for a series of substituted benzoic acids in acid and alkaline media to show the relationship between structure and phosphorescence. In general, the experimental observations have shown that phosphorescence lifetimes and intensities fall into series that relate to the pi electron distribution. It would appear, therefore, that phosphorescence intensity and lifetime data can be used for characterization.

On the other hand the data obtained could be used to predict phosphorescent behavior.

The application of nuclear magnetic resonance to the development of a practical flowmeter has progressed in the direction of improved electronic methods for the reproducible high sensitivity detection of the proton signal. Improvement in sensitivity makes it possible to show flow effects in a 2 mm tube and the detection of trace quantities of paramagnetic salts.

The electronic refinements developed in the course of the flowmeter application are equally applicable to detection of paramagnetic indicators and susceptible nuclei for application to, as yet, undefined problems.

LABORATORY OF CARDIOVASCULAR PHYSIOLOGY

The activities of the Laboratory of Cardiovascular Physiology during 1960 included the initiation of several new and interesting lines of investigation, but it was predominantly a year in which previously initiated, long range projects were brought to fruition. The information gathered has proven to be meaningful and has permitted a considerable degree of systematization and generalization.

Homeometric Autoregulation in the Heart

One type of intrinsic response exhibited by the isolated heart is the well known Frank-Starling mechanism. This endows the ventricles with performance characteristics such that the heart ejects whatever volume is put into it. If inflow is augmented and end diastolic pressure and fiber length are thus increased, the ventricle contracts more forcefully and expels an augmented stroke volume. This occurs on a beat-to-beat basis and may be designated as heterometric autoregulation. This year an intensive study of a second type of autoregulation in the isolated heart, one which apparently does not utilize the Frank-Starling mechanism, was initiated. Homeometric autoregulation, as we have called it, requires at least a few beats to develop fully after an increase in activity (the tension developed per unit of time). The ventricle then exhibits performance characteristics of such a character that its end diastolic pressure and fiber length tend to be maintained at or near the initial level because of an increase in myocardial contractility. The intrinsic ability of the ventricle to rearrange its contractility in this fashion is construed to be a matter of substantial importance in the understanding of cardiac function. Current studies are designed to determine whether, when homeometric autoregulation occurs, a net loss of potassium from the heart takes place and whether this can be reasonably construed as being related to the increased contractility.

Studies on the Atrium

Mitral Valve Closure. Further evidence was acquired to indicate that mitral valve closure can be made to occur solely as the result of atrial activity. Data were also obtained indicating that the contractility of the atrium, rather than the level of blood flow, is the primary determinant when atrial activity produces mitral valve closure.

The Relation Between Mean Left Atrial Pressure and Left Ventricular End Diastolic Pressure. Since, at a constant level of sympathetic activity, the stroke work of the ventricle is a function of its end

diastolic pressure (fiber length), and since the mean atrial pressure constitutes the central pressure which must be exceeded by returning venous blood, the level of mean atrial pressure necessary to produce any given ventricular end diastolic pressure was submitted to examination. It was found that sympathetic stimulation lowers and parasympathetic stimulation substantially elevates mean left atrial pressure relative to the left ventricular end diastolic pressure. The change with parasympathetic stimulation occurs without any observable effect on ventricular contractility and is the result of alteration in atrial function. These studies have been extended to man by the Cardiology Section.

Studies on the Catechol Amine Metabolism of the Heart. The observation that cardiac sympathetic nerve stimulation regularly produces measurable increases in the concentration of catechol amine in coronary venous blood was substantiated. With the use of the trihydroxy indole method, the coronary venous output of catechol amine was shown to be a function of the stimulation frequency used.

It was determined that di-isochloroproteranol, which renders the heart unresponsive to cardiac sympathetic nerve stimulation, does not do so by blocking the release of norepinephrine for the latter was readily recovered in large amounts in coronary venous blood under such circumstances. By implication, therefore, the block must occur further down the line.

It was determined that the initial pressor response to bretylium tosylate, a recently introduced antihypertensive agent, can be attributed at least in part to an initial release of catechol amine from the heart, since in the minutes after the administration of this agent, increased amounts of catechol amine were found in coronary venous blood. This finding coincided in time with an observed positive inotropic and chronotropic myocardial effect.

Studies on the Peripheral Circulation

The Effect of Carotid Sinus Activity on Local Oxygen Consumption at Rest and During Exercise. Whereas an extremity at rest exhibits a slight decrease in its oxygen consumption when carotid pressure is lowered, the same intervention will substantially increase oxygen consumption in that extremity if it is exercising. This important finding is in line with the view that a major physiologic responsibility of the baroreceptor is to safeguard the biochemical status of peripheral tissues as their activity varies. The pathways (effect on atrial and ventricular function) by means of which this is at least partially accomplished is outlined in last year's annual report and current publications.

The effects of direct sympathetic nerve stimulation indicate that the increased flow in the exercising limb results from a functional sympatholysis, which is a graded curvilinear function of the local oxygen consumption.

Experiments employing the cannulation of lymphatics and the collection of lymph from the lower extremities of dogs at rest and during electrically induced exercise have been begun with a view to ascertaining whether an increase in kallikrein activity occurs in the perivascular space during exercise to account at least partially for the observed functional sympatholysis. This study is still in its early stages.

Studies on the Kallikrein System

Further investigation of the pharmacologically active hypotensive proteinases called kallikreins have revealed that the kallikreins derived from a variety of sources hydrolyze those synthetic substrates which contain arginine as the specific amino acid residue. The major portion of the esterase activity in the urine of normal man, as measured by its ability to digest p-toluene-sulfonyl-L-arginine methyl ester, is due to kallikrein. Attempts to differentiate plasma kallikrein from the permeability factor of Miles and Wilhelm or the glass-activated proteinase have been unsuccessful. On the other hand data have been obtained which would suggest that the plasma of patients deficient in Hageman factor are not deficient in kallikreinogen, but rather lacking in kallikreinogenase or some prior enzymatic step required for the activation of plasma kallikrein. Partially purified human urinary kallikrein has been shown to be antigenic in rabbits and the antibody is capable of inhibiting the vasodilator activity of several of the kallikreins. Two active polypeptides, called Kallidin I and II, have been isolated from human plasma following the action of human urinary kallikrein. Methods have been developed during the year which have made it possible to purify human urinary kallikrein an additional 20-fold; human pancreatic kallikrein, 10-fold; and human plasma kallikrein, 30-fold.

Studies on Reflex Factors Influencing Renal Vascular Resistance

The diuresis observed during stimulation of the stellate ganglion led to a series of studies concerning the effect of carotid sinus stimulation on renal vascular resistance. Although this problem has been approached by numerous investigators, the reported data have seemed to be more confusing than enlightening.

Experiments were designed to determine not only whether an increased renal vascular resistance occurs during carotid hypotension but

also whether such a resistance increase is exclusively intrinsic (autoregulatory) or is, to an appreciable extent, attributable to sympathetic stimulation. In lightly pentothalized dogs, occlusion of both common carotid arteries produced an increase in calculated renal vascular resistance as measured by clearance techniques. A method was then designed by means of which the blood flow through one kidney could be directly metered without producing any ostensible disturbance of the renal innervation. This was accomplished by hooking an angled occlusive cannula into the lumen of one renal artery after introducing it from below through an iliac artery and then perfusing that kidney at a metered, constant flow before and during carotid artery occlusion. During carotid hypotension renal arterial pressure rose markedly, the increment sometimes exceeding that in the aorta. The responses in this type of experiment have been consistent; sympathetic impulses, reflexly engendered by the carotid sinus, produce substantial changes in renal vascular resistance and blood flow. This study, among other things, suggests that the consequences of a change in carotid sinus pressure itself can influence blood volume.

Studies on Pulsus Alternans

Alternating End Diastolic Fiber Length as a Causative Factor. The variety of techniques and preparations now available makes possible a comprehensive and sophisticated analysis of myocardial phenomena. One example in point is the definitive analysis of pulsus alternans. Observations on this phenomena have been made in the dog and cat papillary strip, the isovolumetric, innervated in situ dog heart and the isolated, supported dog heart. The data obtained are not consonant with the most popularly held explanations, namely a) a primary alternating impairment of contractility from beat to beat; b) an alternating deletion of fractionate contractions, and c) an alternation of the excitation mechanism. The data do indicate that, when pulsus alternans occurs, the weaker beat (WB) can be accounted for on the basis of a shorter initial fiber length from which the WB initiates. The shorter fiber length prior to the WB is due to an inadequate period for diastole; this does not allow time for complete relaxation and/or a comparable period for filling. This limited diastole is due to the large percentage of the total cycle time (larger stroke volume) required for systole in the previous, stronger beat (SB). The WB has a shorter systolic period due to its smaller stroke volume. This allows a longer period for relaxation and filling and thus a longer initial fiber length to be acquired prior to the SB. Thus pulsus alternans occurs when myocardial contractility is continuously depressed relative to the existing heart rate and stroke volume. When, under these circumstances, contractility is increased (shortening of systole), as by norepinephrine, stellate stimulation or homeometric autoregulation, pulsus alternans abates.

Studies on Chemoreceptors

The Carotid Body. This laboratory has made a major research investment in the investigation of baroreceptors as sense organs which reflexly influence the circulation. More recently attention has been turned to the chemoreceptors. Investigations of the reflex effects upon the heart and systemic circulation of stimulation of the carotid bodies with hypoxic and hypercapnic blood were made in the open-chest dog. The carotid sinus-body area was independently perfused using a double disc oxygenator system which permitted rapid change from blood of one gas composition and pH to that of a different composition and pH without changing perfusion pressure. Continuous measurements of the pO_2 (polarographic) and pH of the perfusing blood were made. Caudal and cephalic blood flow were separately measured utilizing a Potter electroturbinometer and a Shipley-Wilson rotameter respectively. Left ventricular end diastolic pressure, aortic pressure and heart rate were simultaneously recorded. Puzzlingly, bradycardia is the primary heart rate response to carotid body stimulation confirming the findings of deBurgh Daly and Scott. Preliminary data suggests that this negative chronotropic effect is due principally to parasympathetic activation but that, in addition to the pathways found by deBurgh Daly and Scott, sympathetic withdrawal may also be present. These data permitted construction of ventricular function curves relating left ventricular end diastolic pressure and stroke work at constant heart rate (electrically paced). The relative effects of increased peripheral vascular resistance and changes in ventricular contractility, and the roles of neural and humoral factors in these responses are being investigated.

The Mechanism of the Rise in Arterial Pressure Following Occlusion of Abdominal Arteries. The rise of arterial pressure following occlusion of the celiac and mesenteric arteries in the cat under chloralose has previously been ascribed, on the basis of previous work in this laboratory, to a pressoreceptor reflex. It now appears that, at least for the time being, this position must be revised. The results of a variety of more recently conducted experiments which included completely isolating the splanchnic vascular bed, both with and without cross perfusion, and the recording of action potentials, indicate that both mechanical and reflex factors are operative. The occlusion of vessels per se increases the peripheral resistance. Also, blood runs off from vessels distal to the occlusion into the active circulation and so tends to increase heart output by acting as an infusion. Pressor reflex effects due to local irritation as ligatures were tightened on arteries were regularly found, but when these were excluded in cross perfusion experiments, lesser reflex pressure changes could still be elicited by arresting the flow in splanchnic vessels.

Whether pressoreceptors, chemoreceptors or ischemic pain receptors are involved remains to be established.

Studies on Active State, Force Velocity Relationships and Inotropic Mechanisms in Mammalian Myocardium. Some progress has been made in the study of isolated strips of heart muscle. The less complicated geometry and the element of control (shortening vs. tension) possible has afforded the opportunity to examine more precisely the kinetics of contracting myocardium with a view to a better understanding of changes in contractility.

Simultaneous length-tension measurements were made in dog and cat papillary muscle. It was found that in myocardium the intensity of active state can increase, the force-velocity curve can be changed, and thus the intrinsic rate of contraction changed in accord with varying conditions, allowing increased force of contraction without systolic prolongation. Force velocity and maximal work curves shifted to the right with increased initial length, increased Ca^{++} and norepinephrine; to the left with increased K^{+} or acetylcholine. Using a modified quick release method (Ritchie), three phases of active state were determined: 1) Intensity, by redeveloped dp/dt. 2) Duration of Full Intensity, by departure of redeveloped dp/dt from initial dp/dt. 3) Decay, by decline of redeveloped dp/dt. Increased initial length, increased Ca^{++} and decreased K^{+} increased intensity without essential change in duration of active state. Increased rate and norepinephrine increased intensity while shortening duration. Decreased temperature (30^{o} — $15^{o}C$) prolonged duration and decay with little change in intensity. Dichloroisoproterenol blocked norepinephrine effects, but not rate staircase or Ca^{++} effects. These data demonstrate that in myocardium, unlike skeletal muscle, variations in the intensity and duration of active state are experimentally dissociable.

Studies on the Relative Effects of Shortening and Tension Development in Heart Muscle. It is hoped that the less complicated geometry of the isolated heart muscle strip will make it possible to gather data on the relation of myocardial oxygen consumption to tension in a situation in which the undetermined variable due to radius of curvature (La Place) in the intact ventricle would not be present. Progress has been blocked by the lack of a sufficiently stable and sensitive pO_2 electrode. It now appears that this instrumentation problem has been overcome.

The mechanism of bicarbonate reabsorption in Necturus is being reinvestigated. It is now generally assumed that bicarbonate reabsorption in both the proximal and distal segments of the kidney is effected by an exchange of hydrogen ions derived from the tubular cells for intraluminal sodium ions with the resultant formation of carbonic acid in the tubule lumen. The carbonic acid is thought to be converted to CO_2 and water, the CO_2 escaping by diffusion. It is implicit in the theory that the rate of formation of CO_2 is equal to the rate of bicarbonate reabsorption. It has been pointed out that the intraluminal concentration of H_2CO_3 necessary to provide the driving force for the non-catalyzed dehydration of H_2CO_3 to CO_2 and H_2O at the required rate would result in an intraluminal pH approximately 1 unit less than that when equilibrium is achieved. Alternatively were the reaction catalyzed by carbonic anhydrase residing in the luminal membrane the in situ pH would not necessarily be depressed but administration of carbonic anhydrase inhibitors should lower rather than elevate it.

It is not possible to evaluate these alternative possibilities by determining the pH of fluid removed from the tubule lumen for measurement, since subsequent equilibration of CO_2 and H_2CO_3 in the measuring receptacle by non-catalytic means would raise the final pH and mask any in situ differences. For these reasons much of the effort in the past year has been directed towards the development of an appropriate micro pH electrode which would permit the in situ determination of pH in the proximal tubule fluid of Necturus. Thus far this has proved to be difficult and only a few pH-sensitive micro electrodes have been developed. In preliminary studies, no clearcut evidence of acidification in the proximal tubule of Necturus has been observed, suggesting that the dynamic pH (in situ) does not appreciably differ from the equilibrium pH. If confirmed, this observation would lend support to the view that the dehydration of carbonic acid within the tubule lumen is catalyzed by carbonic anhydrase residing in the tubule cell membrane.

Certain aspects of phosphate transport in the dog kidney, a process intimately related to urinary acidification, are being reinvestigated. Although net phosphate secretion in the chicken has been previously reported from this laboratory, it had generally been agreed that phosphate is not secreted in either dog or man. In recent years, however, several studies have appeared in the literature in which net secretion of phosphate was reported to have occurred in the dog. Since in none of these studies was the filtered phosphate load maintained at a reasonably constant level, the evidence is inconclusive. In order to establish the presence or absence

of net phosphate secretion in the mammal, the renal excretion of phosphate is being studied under steady state conditions in a variety of experimental situations which include most of those previously reported to have been associated with the observation of net phosphate secretion. Neither prior administration of parathormone for a variable period of time, prior administration of parathormone and superimposed mannitol diuresis, prior loading with sodium phosphate for three days before study, or acute lowering of the glomerular filtration rate, has resulted in the excretion of phosphate in excess of that filtered.

An investigation of the effects of the infusion of ammonium salts on electrolyte excretion in the kidney has been completed. It had been observed that the administration of ammonium chloride into the renal portal venous circulation of the chicken resulted in a predominately unilateral increase in sodium chloride and water excretion on the injected side, as well as a rise in the excretion of ammonia. This effect has been shown to be due to the ammonium ion itself, since it occurs with the ammonium salts of sulfate, acetate, nitrate, as well as chloride. Equivalent acidosis produced by hydrochloric acid does not result in similar changes in urinary composition. The natriuresis is also independent of the rate of ammonia excretion. It occurs when the ammonia excretion is minimized by prior alkalinization of the urine. Although in all studies net potassium secretion diminished in association with the fall in sodium reabsorption, no change in net hydrogen ion transport was observed. The mechanism of the response to ammonium salts is unclear. It is possible that the effect is secondary to interference with potassium uptake by the renal tubule cells. NH_4^+ ion may substitute for K^+ on the contraluminal Na-K exchange pump. It should be noted that substitution of ammonium ion for potassium on the sodium-potassium exchanger has been suggested on the basis of analogous studies in the red cell in other laboratories.

A number of studies of the mechanism of urinary dilution and concentration are in progress. The effect of one of the benzothiadiazine diuretics, chlorothiazide, on urinary dilution and concentration in the dog has been investigated. These agents diminish the rate of free water excretion during water diuresis in both man and dog. It has been assumed that this is a consequence of interference with the reabsorption of sodium and chloride in a water-impermeable segment of the renal tubule. No analogous studies during the elaboration of hypertonic urine have been reported. The present studies have also indicated that the decrement in solute-free water excretion when chlorothiazide is administered can be quantitatively accounted for by the increment in NaCl excretion suggesting that the major site of action of this drug is at the site within the renal tubule at which removal of sodium and chloride leads to dilution of the urine.

The relationship between negative free water and solute excretion over a wide range of solute excretion has been examined in hydropenic animals pretreated with vasopressin. Results obtained during the administration of chlorothiazide have been compared with those during the injection of non-absorbable solute such as mannitol. At low rates of solute excretion, negative free water clearance is unaltered by chlorothiazide. In contrast, at high rates of solute excretion, a uniform increase in negative free water clearance occurs when chlorothiazide is administered. Since massive solute diuresis induced by non-pharmacologic agents is associated with a progressive fall in negative free water clearance, the rise observed with chlorothiazide under these circumstances may be indicative of the delivery of a more concentrated solution from the distal convolution to the terminal concentrating site. In this view the drug does not exert a primary effect on the terminal concentrating mechanism.

The effects of this agent in patients with nephrogenic diabetes insipidus have also been examined. It had previously been reported that the benzothiadiazine diuretics not only increased urine osmolality in patients with diabetes insipidus but also diminished urine flow. Although the increase in urine osmolality is clearly the result of a diminution in sodium transport in the distal convolution (see above) this single action can not account for the associated antidiuretic response. Oral administration of either chlorothiazide or hydrochlorothiazide to four patients with vasopressin resistant diabetes insipidus resulted in a uniform increase in urine osmolality though never to a concentration exceeding that of plasma, and a 30-40% diminution in urine flow. The effect was considerably greater when the patients were maintained on a low sodium intake than when maintained on a high sodium intake. Furthermore, the antidiuretic response could be maintained despite withdrawal of the drug if the restricted sodium intake was continued throughout the course of the study. A reversal to the polyuric phase was uniformly observed when sodium was readministered in the diet. On the basis of these observations, it has been suggested that antidiuresis produced by chlorothiazide is a consequence of a reduction in body sodium content. The fall in sodium content reduces volume flow to the distal portion of the nephron either by a reduction in filtration rate or by an increase in proximal reabsorption. If this interpretation is correct, salt depletion produced by other agents should also result in an antidiuretic response in these subjects. This possibility is being examined at present.

In association with the above studies, the effect of chlorothiazide has been examined in adrenalectomized rats. It has previously been suggested by others that the antidiuretic and chloruretic effect in diabetes insipidus is secondary to interference with the renal action of aldosterone. The conclusion is based largely on the report that chlorothiazide is ineffective in adrenalectomized rats. In contrast, the present studies indicate

that the diuretic response does not differ in normal animals and in adrenalectomized animals maintained on an adequate salt intake.

Further experiments relating to the concentrating mechanism are being pursued in an attempt to develop a method for the estimation of renal medullary blood flow in the intact animal. It has been established that medullary and papillary tissue of the kidney is hypertonic to plasma. The maintenance of this concentration gradient within the medullary and papillary tissue is dependent upon the unique anatomical arrangement of the vascular tree within the deeper portions of the kidney, sequestered solute being effected by countercurrent exchange of water and solute between the opposing limbs of the vessel. In order to measure one of the variables affecting the efficiency of the countercurrent exchanger and thus the concentration of the urine, the possibility of applying a modified Fick principle for the estimation of medullary flow is being investigated utilizing hydrogen as an indicator.

Net water movement and the unidirectional fluxes of tritiated water through a mesityl oxide membrane have been measured. In contrast to prediction, the flux ratio of water through mesityl oxide, a presumably non-porous organic membrane, deviates significantly from the activity ratios. The possibility of droplet movement through the mesityl oxide has been eliminated by comparing the flux of sodium^{24} with that of water. Since it is apparent that neither active transport of water nor bulk flow occurs through this membrane, the discrepancy between the flux ratio and activity ratio is inexplicable though it points up the difficulty of accepting the usual criteria for bulk movement at face value. Preliminary measurements of the water content of the mesityl oxide equilibrated with water of various activities have indicated a significant departure from linearity between the water activity and the water content of the equilibrated mesityl oxide. If this non-linearity is assumed to persist at the two interfaces of the model membrane this may explain the unexpectedly high flux ratio observed.

Studies of water and solute movement through toad bladder have been initiated using isolated halves of toad bladder suspended as closed sacs in test solutions. In the absence of vasopressin net water movement in response to an osmotic gradient established with sodium chloride, mannitol or urea, is approximately equal. In the presence of vasopressin, however, net transfer of water from a dilute urea solution within the bladder is greater than from comparable solutions of mannitol or sodium chloride. Furthermore, net water gain into hypertonic solutions of urea is less than into equivalent concentrations of mannitol or sodium chloride. These differences are dependent upon the associated rapid penetration of urea through the bladder wall presumably via aqueous pores in contrast

with the minimal penetration of either mannitol or sodium chloride.

Studies concerning the mechanism of active cation transport in human red blood cell ghosts have been continued, correlating the activity of the sodium pump with the activity of a specific membrane, "ATPase." The latter is presumed to be involved in some fashion in the active transport of cations. The effect of various substrates and other agents on the ATPase activity (as measured with isolated membranes permeable to ATP) has been compared with active sodium transport (measured in reconstituted ghosts). It has been observed that both sodium extrusion, an estimate of pump activity, and ATPase activity, specifically require ATP. Other high energy phosphate compounds were ineffective in both systems. Both sodium transport and ATPase activity were inhibited by sodium fluoride and copper chloride, but neither was affected by iodoacetate, sodium azide or sodium arsenate. The action of strophanthidin was similar in both systems. Both systems require magnesium and calcium inhibits both the ATPase activity and sodium extrusion. These comparative studies provide further evidence in support of the view that an ATPase-like enzyme residing within the cellular membrane is involved in the active transport of sodium and potassium across the red cell wall.

The characteristics of an ATPase isolated from the renal cortical tissue of dog, guinea pig and rat are being studied. Thus far the studies are in a preliminary form and the precise conditions for maximal activation of the enzyme system have not yet been developed.

A reliable automatic coulometric-amperometric titration method for chloride analysis has been developed; in the past year the method has been further improved. The analysis of chloride in a large variety of biological fluids has been undertaken in order to demonstrate its validity as a useful biochemical method. It has been applied in a project designed to characterize the distribution of chloride across tissue cells in nephrectomized rats. According to the simplified Conway hypothesis, appreciable amounts of chloride should shift into the muscle cell in the presence of hyperkalemia and acidosis. To test this hypothesis the distribution of chloride and of isotopically labeled inulin has been compared in nephrectomized rats with severe acidosis and hyperkalemia. In the two studies completed the ratio of inulin to chloride spaces was .95 in the hyperkalemic animal, a value not significantly different from that observed in muscle tissue of normal rats. According to the Conway hypothesis the ratio should have fallen to about .75 in the presence of the observed degree of hyperkalemia.

An analysis of the myocardial chloride content in experimental cardiac failure in the dog has been completed. The increment in water

and chloride content in heart muscle of dogs with congestive heart failure can be accounted for solely on the basis of an expansion of the extracellular space of this tissue. It is apparent that these alterations in water and electrolyte content do not constitute the primary defect responsible for the heart failure though they may in all probability contribute to a further depression in myocardial function.

An infinite thickness liquid counting method utilizing the gas flow detection chamber has been developed in order to facilitate the estimation of C^{14} labeled inulin. Data obtained using this method have confirmed results obtained in this laboratory a number of years ago which provided evidence that inulin clearance does not vary with changes in plasma inulin concentration; the use of radioactive inulin has extended the range of plasma concentration an additional order of magnitude lower.

Investigation of the role of a cardioglobulin system in mammalian plasma has been continued. It had previously been reported that cardioglobulin exerts a positive inotropic effect on the isolated frog heart. Using this as an assay it has been shown that the cardioglobulin content of human plasma is elevated in hypertensive disease, markedly depressed in some patients with idiopathic myocarditis, and may be acutely lowered during the course of extracorporeal perfusion of blood. The present efforts are directed at determining the role of this protein system in the mammal. For this purpose the cardiovascular effect attendant upon depletion of cardioglobulin in the rat has been assessed. Chronic cardioglobulin depletion by exchange transfusion has proved virtually impossible, as a result of the extreme rapidity of the regeneration of the protein. Although it has been possible to demonstrate an acute diminution in cardioglobulin concentration in rat plasma, a return to normal values occurs within 24 hours. The acute cardiovascular effects of administration of cardioglobulin-free plasma to the rat have thus far proved to be negligible, in part due to methodological difficulties. In acute studies administration of cardioglobulin rich plasma to the failing rat heart-lung preparation does not affect cardiac performance as estimated by alterations in heart rate, blood pressure, blood flow through a shunt and venous pressure. Further experiments are planned in order to verify this conclusion.

The mechanisms regulating the secretion and metabolism of aldosterone in dogs with secondary hyperaldosteronism have been studied. A reexamination of the role of the arterial baroreceptor in the control of aldosterone secretion has been completed. On the basis of these studies, it may be concluded that the baroreceptors in the aortic arch and the carotid arterial tree are not essential to either the hypersecretion of aldosterone or the almost complete sodium retention observed in dogs with inferior vena caval constriction, and further, that marked reductions

in pulse pressure in the carotid arterial system and in the mesenteric arterial tree fail to increase the rate of aldosterone secretion. More recently, in collaboration with Drs. Anderson, Haymaker and Spense of NIAMD, a role of the midbrain in the regulation of aldosterone excretion in the same experimental preparation has been excluded. Thus, following midbrain transaction, acute hemorrhage results in a striking increase in adrenal vein aldosterone secretion in dogs with vena caval constriction. The response does not differ appreciably from that observed in vena caval dogs with intact midbrains. It has also been shown that the adrenal and kidney transplanted to the cervical region, though devoid of nervous connections, respond in the "normal" fashion to acute hemorrhage. Aldosterone secretion increases and sodium retention supervenes in the cervically transplanted organ, despite an absence of both nervous connections and an increase in venous pressure in these organs.

The most interesting observation made in the past year in relation to this problem has been the demonstration that the kidney releases an aldosterone stimulating hormone which signals the release of mineralocorticoids from the adrenal. In the absence of ACTH, enhanced secretion of aldosterone from the adrenal in dogs following acute hemorrhage is considered presumptive evidence for secretion of aldosterone secreting hormones. In a series of successive ablation experiments, removal of the anterior pituitary, removal of the head, of the liver, etc. was generally followed by an increase in aldosterone secretion in response to acute hemorrhage. In contrast, removal of the kidneys prevented the increase in aldosterone secretion generally attendant upon acute blood loss. Furthermore the intravenous infusion of saline extracts of kidney resulted in a marked increase in aldosterone production. These data provide conclusive evidence for the renal origin of an aldosterone stimulating hormone.

The aldosterone stimulating hormone derived from kidney does not appear to be renin. Experimental hypertension in the dog is not associated with increased rates of aldosterone secretion. Renin administration does not augment aldosterone secretion although injection of hypertensin II may in some instances stimulate its release. An associated increase in corticosterone secretion due to hypertensin II, not generally seen following hemorrhage in vena caval dogs, suggests that the response to hypertensin II is not analogous to that due to aldosterone stimulating hormone of renal origin. More recently it has been shown that there is a marked fall in aldosterone secretion following nephrectomy in hypophysectomized dogs with constrictions of the inferior vena cava. This is additional support for the role of the kidney in the elaboration of a tropic hormone.

Amine Biogenesis and Metabolism

It is now certain that there is one enzyme, designated as aromatic L-amino acid decarboxylase, that is responsible for decarboxylation of all the normally occurring aromatic L-amino acids including 3, 4-dihydroxyphenylalanine, 5-hydroxytryptophan, tryptophan, tyrosine, phenylalanine, histidine and kynurenine. Substrate-enzyme studies have been carried out for all these amino acids. In addition to this general amino acid decarboxylase there is also found a specific L-histidine decarboxylase. This means that there are at least two different catalysts for converting the dietary amino acid histidine to the potent pharmacologic agent histamine. In comparing the two mechanisms for histidine decarboxylation, it was found that the specific enzyme is present in tissues that are rich in mast cells (mast cell tumors); it acts only on L-histidine, has the higher affinity for histidine (K_m 10^{-4}M) and has a pH optimum near 6. It is not inhibited by -methyl DOPA. By contrast histidine decarboxylation by L-aromatic amino acid decarboxylase occurs in organs containing few mast cells, the K_m is nearer 10^{-2}M, pH optimum is about 8.5 and the activity is inhibited by low concentrations of -methyl DOPA. The significance of these two separate mechanisms for histidine decarboxylation is not apparent. However, it has been suggested by Dr. Richard Schayer, that there is a marked increase in histidine decarboxylase in some tissues (up to 10 fold) during conditions of stress. Studies on the mechanism of this apparent enzyme induction are now in progress. Inhibitors of aromatic L-amino acid decarboxylase were studied further and it has been found that the two most potent ones, -methyl DOPA and -methyl metatyrosine, have two actions in animal tissues in vivo. The first action apparently results from its known enzyme-inhibiting activity and leads to a fall in the serotonin and dopamine concentration of brain. However, 24 hours after a single dose of about 100 mg/kg the concentration of these two amines has returned to normal. On the other hand, the concentration of noradrenaline in heart and brain falls to values less than 10% of normal and remains low for periods of several days. Various experimental procedures indicate that the -methyl amino acids prevent the binding of noradrenaline by tissues. It would appear, therefore, that they act in a manner similar to reserpine with the important difference that their effects are limited to noradrenaline. This property makes them extremely valuable as tools in studying the functions of brain amines since it is possible to prepare animals which have normal amounts of brain serotonin and dopamine but little noradrenaline. This is essentially the situation 24-30 hours after a single dose of -methyl metatyrosine in mice, rats, guinea pigs and rabbits (100-200 mg/kg). It is of interest that after

an initial short period of apparent sedation the animals appear perfectly normal. Studies on the mechanism of the antihypertensive action of these agents in man by the Section on Experimental Therapeutics are complementary to those in this laboratory. In addition, members of the Laboratory of Chemical Pharmacology have used the α-methyl amino acids in their studies on the physiology of the amines.

One of the consequences of the existence of a nonspecific L-amino acid decarboxylase is that amine derivatives of all of the normally occurring amino acids should be formed. This appears to be the case and phenylethylamine, tyramine and tryptamine have been shown to be excreted in the urine. Ortho and meta tyramine are also found although it is not yet known how they are formed. Other interesting amines which have been found in urine are synephrine and norsynephrine (p-hydroxyphenylethanolamine). The former, which may be considered as adrenaline with one less ring hydroxyl group, is found in urine in amounts which are comparable, and frequently greater, than that of the normetanephrines. Since synephrine is a fairly active pharmacologic agent, its presence in such amounts may have physiologic significance. However, of more biochemical interest was the indication that the side chain oxidation, such as occurs in the conversion of dopamine to noradrenaline, can occur with other compounds. It was subsequently found that purified preparations of the enzyme, dopamine-β-oxidase, oxidize tyramine to norsynephrine and that the enzyme is therefore not specific for dopamine. The finding of the N-methylated compound in urine indicates that the enzyme noradrenaline N-methylpherase is also nonspecific. It appears, therefore, that most of the enzymes involved in aromatic amine biogenesis are nonspecific. This nonspecificity explains the presence of the numerous aromatic amines and their metabolites in urine.

An important route of metabolism of serotonin leads to the pineal gland hormone, melatonin (N-acetyl-5-methoxytryptamine). In conjunction with members of NIMH it has been shown that in the pineal gland serotonin is first acetylated and then methylated to yield melatonin. The two enzymes have been partially purified and studied. The methylpherase is specific and is found only in the pineal gland. Melatonin which leaves the pineal gland is metabolized in the liver by the microsomal hydroxylating system to yield 6-hydroxymelatonin.

Amine metabolism is also carried out by nonspecific enzymes and many products are to be expected in urine. Methods for the assay of the o-methylated metabolites of the epinephrines and of their acid end product, 3-methoxy-4-hydroxymandelic acid, have been developed. These are specific and precise. Assays of a similar type are applicable to norsynephrine and p-hydroxymandelic acid. All these assays are now being used in collaboration with the Section on Experimental Therapeutics to

study these amine pathways in man, normally and in various disease states. In the case of noradrenaline two major routes of metabolism are possible, oxidative deamination and o-methylation. Studies on animals in vivo indicate that only in the liver is o-methylation the major route of noradrenaline metabolism. In brain, heart, and other organs the major route is oxidative deamination. This means that noradrenaline synthesized and released within an organ is metabolized by monoamine oxidase, only that fraction which leaks out into the blood is methylated on passage through the liver.

The enzyme, monoamine oxidase (MAO) has now been obtained in soluble form from mitochondria and has been purified at least 10 fold from this source. This represents a considerable advance in purification. It has been found that the purified enzyme splits N-dimethyl amines into the corresponding aldehyde and dimethylamine and that N-dimethylamine oxides are deaminated in the absence of oxygen. It may be that MAO catalyzes a direct oxidative attack on the nitrogen and that the N-oxides are intermediates. While a few N-oxides have been isolated from natural sources no enzymatic studies of this type have ever been carried out. Other oxidative deaminases such as L-amino acid oxidase, D-amino acid oxidase and diamine oxidase are really dehydrogenases, the oxygen coming from hydrolysis of an intermediate imino compound. In the case of MAO, oxidation must then occur through atmospheric oxygen. Experiments with O^{18} are now in progress to verify this point.

Choline Biogenesis

The biogenesis of choline has been studied in animals. Although serine is the ultimate precursor it was not possible to demonstrate direct decarboxylation of this amino acid to yield free ethanolamine. These studies indicated, rather, that serine is incorporated into phospholipid and in this form is decarboxylated to phospholipid-ethanolamine (cephalin) and, still in lipid form, is methylated through intermediate mono and dimethyl forms to phospholipid choline (lecithin). All the methyl groups arise from the methyl groups of methionine. Utilization of one carbon sources such as formate or formaldehyde occurs only by prior conversion to methionine methyl groups. Overall synthesis of choline from serine, in phospholipid form, also follows from the work of Dr. David Greenberg and collaborators at the University of California. Such a pathway may have great physiologic significance. Conversion of a dimethylamine to a quarternary amine in a phospholipoprotein structure would be a most effective way to alter the charge on a membrane and thereby regulate transport mechanisms. In liver the entire series of reactions occurs in the microsomal portion of the cell. It may be that they are involved in determining the lamellar structure of the endoplasmic reticulum.

γ-Aminobutyric Acid Metabolism

Further studies on γ-aminobutyric acid (GABA) metabolism were carried out. The major work has been on the identification of the GABA-histidine containing peptide in brain as γ-aminobutyryl histidine (homocarnosine). It represents the GABA analogue of carnosine. The latter is found mainly in muscle and other peripheral organs and does not occur in brain. Homocarnosine is found only in the central nervous system and urine. Methods for assay of the several carnosines have been developed and brain levels have been measured in many animal species. In man, monkey and cattle, brain contains as much as 30 μg/gram. Although this is considerable it represents only 5% of the total GABA content. The unique localization would appear to be indicative of the availability of β-alanine or GABA in the various tissues rather than of enzyme specificity. When significant GABA levels were maintained in peripheral organs (by including GABA in the diet) appreciable amounts of homocarnosine appeared in muscle. Normally it is not found there. These findings corroborate experiments of Meister concerning the lack of specificity of the enzyme, carnosine synthetase. The significance of homocarnosine in brain remains to be determined. Its presence in urine may provide another measure of central nervous system chemistry since it is normally formed only in brain.

Amino Acid Transport

Studies on the uptake of aromatic amino acids by various tissues in vivo and in vitro have been continued. L-Tyrosine uptake by brain in vivo unquestionably involves a mechanism of facilitated transport. It has now been found that it has a most rigid stereospecificity. In previous studies it was found that following administration of D-tyrosine some tyrosine appeared in brain, though much less than with the L-isomer. It has now been found that the tyrosine entering brain after administration of D-tyrosine is all in the L-form indicating that racemization had taken place before penetration into brain. The D-form does not pass the "blood brain barrier." Little or no distinction between uptake of D and L-tyrosine occurs in other tissues. These and other findings suggest that the same anatomical area which is considered as the "blood brain barrier" also possesses mechanisms to facilitate the transport of essential metabolites of the central nervous system. Such findings make it necessary to point out what should have been obvious before this, that the "blood brain barrier" theory requires the existence of mechanisms of active transport at the same site, to explain the uptake of metabolites with physical properties comparable to those which are usually prevented from penetrating. Although much of the mechanism for facilitated transport of L-tyrosine resides in the "blood brain barrier" and can be demonstrated only in the

intact animal, brain slice uptake of tyrosine is also unique. Brain slices concentrate tyrosine as much as 3 fold over the incubation medium. To do this they require an energy source which can be supplied by glucose, mannose and related compounds. Metabolic poisons inhibit this uptake. Muscle, liver, spleen and kidney slices do not concentrate tyrosine. In rat diaphragm muscle all the uptake of L-tyrosine can be explained on a diffusion mechanism. These differences between brain and other tissues in uptake of L-tyrosine no doubt extend to many other amino acids and metabolites and it will be of interest to examine other metabolites in the same way.

Mechanisms of Aromatic Hydroxylation

Some additional studies have been carried out on the mechanism of aromatic hydroxylation. Using tritium labelled phenylalanine (ring) and T_2O it was found that the para position of phenylalanine is not labilized in the presence of phenylalanine hydroxylase. An intermediate substrate activation should have yielded exchange between the para hydrogen of phenylalanine and water. This did not occur. Similar studies are being started with acetanilide labelled with tritium in the para position. In this case microsomal aromatic hydroxylase will be used.

Collagen and Hydroxyproline

Studies on collagen biosynthesis and hydroxyproline formation have been extended. The presence of rapidly turning over forms of collagen, suggested by studies on urinary hydroxyproline have been confirmed by direct studies on tissue collagen. This confirms our previous conclusion that although body collagen as a whole is metabolically inert there are small pools of collagen which enter into the same "dynamic states" as other tissue constituents. Some of these findings are being explored in man by the Section on Experimental Therapeutics. Studies on chick embryos have shown that collagen (hydroxyproline peptide formation) occurs in microsomes and its requirements are similar to those of other protein syntheses. In preliminary studies it has been possible to demonstrate "collagen" formation in vitro in microsomal material isolated from chick embryos.

Studies have been initiated to investigate the metabolic relationship between proline, hydroxyproline and ketoproline found in the various actinomycin molecules. Micro-procedures for the isolation of the different actinomycins were devised. In an initial study with C^{14} labeled L-proline it was found that the actinomycins synthesized by washed suspension of S. antibioticus were isotopically labeled. Moreover, hydroxyproline was found to contain appreciable radioactivity. Formation of the actinomycin

chromophore, 2-amino-4, 6-dimethyl-3-phenoxazinone-1, 9-dicarboxylic acid, has been achieved using cell-free extracts of S. antibioticus and 3-hydroxy-4-methylanthranilic acid as substrate. In addition, the cell-free system will condense a number of other ortho-aminophenols for example, 3-hydroxykynurenine, 3-hydroxy-4-methylanthranilic acid methylester and 3-hydroxyanthranilic acid to form phenoxazones. The purification and properties of this enzyme system are now under investigation. It may be possible to achieve an overall enzymatic synthesis of the proline and hydroxyproline actinomycin chromopeptides.

GENERAL MEDICINE AND EXPERIMENTAL THERAPEUTICS BRANCH

Section on Clinical Endocrinology

The research program of the Section on Clinical Endocrinology has included studies on 1) aldosterone metabolism in edematous states, 2) calcium metabolism in metabolic bone disease, 3) renal function, with special reference to free water clearance, and 4) experimental atherosclerosis.

1) Studies on aldosterone metabolism included clinical studies on the role of aldosterone in fasting, in primary and secondary aldosteronism, in idiopathic edema, and in potassium depletion, both spontaneous and experimentally induced. The role of the kidney and of various pressor agents in the regulation of aldosterone secretion has been explored in dogs.

It was confirmed that the sodium loss with fasting is greater than that with sodium deprivation alone; in some patients this was associated with failure of aldosterone secretion to rise with salt loss, but these patients lost potassium with fasting, and the relative hypoaldosteronism may have been a result of this. Eight patients with primary aldosteronism were studied before and after surgery. The use of a low sodium diet preoperatively allowed restoration of potassium deficits and an increase in aldosterone secretion. The same result was seen with aldosterone antagonists, which thus might increase aldosterone secretion in primary aldosteronism (by promoting potassium retention) as well as in secondary aldosteronism (by inducing sodium loss).

The expansion of intravascular volume with albumin, on the other hand, did not lower aldosterone secretion in primary aldosteronism as it does in secondary aldosteronism. Direct measurements of blood volume in patients with edema showed an inverse relationship of intravascular volume and aldosterone secretion; a similar inverse relationship related arterial pulse pressure and aldosterone secretion. Methodology for determination of aldosterone was improved by introduction of C_{14} aldosterone biosynthetically prepared, and of a "visible" aldosterone marker. Studies on gas chromatography of aldosterone were initiated.

A study of the possible role of diurnal rhythms in the cause of edema was instituted by extensive "mapping" of diurnal rhythms in normal female subjects. Aldosteronism was found associated with hyperplasia of juxtaglomerular apparatus in a normotensive boy, and the role of the kidney in control of aldosterone secretion was studied in extenso in dogs.

Nephrectomy was found to reduce or abolish the response of aldosterone secretion to caval constriction in the hypophysectomized dog. Contrary to reported findings, this response was found to occur in hypophysectomized dogs both with and without suprapontine brain removal. Hypertensin (Angiotensin II) and renin were shown to induce aldosterone secretion in the hypophysectomized, nephrectomized animal, and did so in some preparations.

2) Studies on calcium metabolism and metabolic bone disease included clinical balance studies in osteoporosis, in renal osteitis, in sarcoidosis, and in hyperparathyroidism, studies on renal phosphate clearance with special reference to parathyroid function, studies of labile calcium in the parathyroidectomized dog, and studies in the metabolism of vitamin D.

Patients with idiopathic osteoporosis (defined as "idiopathic" by an absence of the response of calcium balance to estrogens or androgens) were studied to ascertain the mode of action of albumin in inducing positive calcium balance and to test the effect of strontium. The possibility of a purely oncotic action of albumin could not be ruled out; strontium appeared to be without effect. Patients with renal osteitis were found to absorb little or no calcium from the gastrointestinal tract, as judged from balance studies and the fecal excretion of orally fed calcium[47]. Vitamin D markedly improved calcium absorption, but aluminum hydroxide did not.

Patients with sarcoidosis were found to absorb abnormal amounts of calcium from the gastrointestinal tract, to increase this absorption further with vitamin D, and to lower it markedly with carbohydrate-active corticosteroids even in the presence of vitamin D. Blood levels of vitamin D were never elevated, suggesting hypersensitivity to, rather than hyperabsorption of, vitamin D.

Renal phosphate and calcium excretion were studied in patients with hyperparathyroidism and in normal control subjects. Normal subjects could not be distinguished from those with hyperparathyroidism on the basis of either maximal reabsorption or clearance of phosphorus, but phosphate excretion in response to a calcium load was abnormal in hyperparathyroidism, as was renal calcium excretion on very low phosphorus intake. Both phenomena could be reproduced in normal subjects with chronic administration of parathyroid extract.

The role of the parathyroids in the renal excretion of phosphate was studied with clearance techniques in dog and man. No evidence for tubular secretion of phosphate could be found, despite phosphorus loading, acidosis, and use of parathyroid extract. Clearcut tubular maxima were

found, and considered to be evidence against the possibility of secretion.

Parathyroidectomized dogs were found to have decreased amounts of readily labile bone calcium, as judged from dynamic response of extracellular fluid calcium to versene-induced hypocalcemia. Parathyroid extract and vitamin D could restore labile calcium to normal and, with higher doses, to above normal. H_3 vitamin D was prepared for us by a commercial firm, and C_{14} vitamin D will be prepared in the near future. Chromatographic systems for isolation of vitamin D have been developed, and in vivo and in vitro studies of its metabolism and fate have been begun.

3) Studies on renal function included measures of free water clearance in patients with edema and in patients with hyponatremia, and studies with Amphotericin B.

Free water excretion was studied in patients with cirrhosis and compared with that in patients with cardiac failure and in normal subjects depleted of sodium. Results indicate that in all these conditions free water excretion is limited, not because there is persistent or excessive secretion of antidiuretic hormone, but because there is excessive reabsorption of salt and water in the proximal tubules. Whereas this appears to be aldosterone-dependent (as judged from the response to aldosterone antagonists) in the patients with cirrhosis as in the normal subjects, it appears not to be aldosterone-dependent in some patients with cardiac failure: in these patients, the filtration fraction appeared to be above normal; catechol amine antagonists had no effect in decreasing proximal sodium reabsorption.

Further studies were carried out in the syndrome of hyponatremia resulting from "inappropriate" secretion of antidiuretic hormone. The syndrome was uncovered in two patients with intermittent acute porphyria. Some fifteen cases with pulmonary or intracranial diseases have been uncovered in various clinics and are the subject of a review in preparation. The syndrome could be reproduced with pitressin in normal subjects, either by deliberate choice of fluid intake or by voluntary ad libitum drinking.

Amphotericin B was found to induce reversible decrease of glomerular filtration rate and renal plasma flow in patients receiving the drug for therapeutic purposes.

4) Studies in experimental atherosclerosis included measures of the rate of penetration of labeled cholesterol and of labeled albumin into the aorta of the dog. It was found that both substances entered more

rapidly in the proximal and progressively less rapidly down the distal aorta. The gradient for albumin does not depend upon pulsatile pressure or upon absolute circumferential tension, but the rate does appear to be pressure-dependent. Further studies, including measurement of lipoprotein in the aortic wall, are in progress.

Section on Experimental Therapeutics

The investigative approach of this laboratory to clinical cardiovascular problems is a combined biochemical-pharmacologic one. For convenience the studies are described under four headings: 1) biogenic amines, 2) amino acid metabolism, 3) action and metabolism of drugs, and 4) miscellaneous.

Biogenic Amines

Using improved methods, the urinary excretion of catecholamines (norepinephrine plus epinephrine), their methoxy-amine metabolites (normetanephrine and metanephrine) and 3-methoxy-4-hydroxymandelic (MOMA) was measured in 23 patients with pheochromocytoma and in a large group of patients with essential hypertension. For purposes of separating patients with pheochromocytoma from the rest of the hypertensive population, a suitable upper limit of normal for each assay was found to be 0.1 mg/day, 1.3 mg/day and 6.0 mg/day respectively. It was determined that the production of catecholamines as indicated by urinary products is not elevated above normal in essential hypertension.

The fact that the excreted methoxy-amines represents a much smaller fraction of the metabolites in normals and hypertensives than does MOMA supports the idea that oxidative deamination is the initial mode of metabolic degradation of that norepinephrine formed at nerve endings. Studies in the rat tend to support this concept. Monoamine oxidase activity of brain and heart was found to be several times that of catechol-0-methyltransferase. Endogenous norepinephrine accumulated when monoamine oxidase was inhibited by drugs; exogenously administered norepinephrine accumulated in the heart under the same conditions. Neither of these effects could be produced by inhibition of catechol-0-methyl transferase.

The vascular response to various sympathomimetic amines is being compared with the effects of these agents on blood unesterified fatty acid (UFA). While norepinephrine alters both variables, equipressor doses of dopamine in patients were found not to provoke an UFA response.

In collaboration with Dr. J. Pisano (LCB), N-methyl-p-hydroxyphenylethanolamine (synephrine) was isolated and identified in human urine. That tyramine may be the precursor of synephrine and norsynephrine is indicated by the finding of elevated amounts of p-hydroxy-mandelic acid in the urine after infusion of tyramine in man. The overall metabolism of tyramine and the physiologic significance of the synephrines are under investigation.

A sensitive and specific chemical assay for histamine in human urine has been developed. Normal values are in the range of 20 to 100 μg/day. In a unique case of the carcinoid syndrome arising from a primary lesion in the stomach, characterized chemically by the excretion of large amounts of 5-hydroxytryptophan, serotonin and 5-hydroxyindoleacetic acid in the urine, urinary histamine was 545-580 μg/day.

Amino Acid Metabolism

It has been established that urinary hydroxyproline (HPr) arises primarily from the breakdown of body collagen, and that the amount excreted is a useful index of collagen synthesis and degradation. There is a progressive decrease in the amount of urinary HPr in successive decades of life, presumably reflecting the "metabolic age" of an individual's collagen. The possible application of this finding to gerontologic problems as well as to selected disorders of connective tissue is apparent.

Studies of the inhibition of aromatic amino acid decarboxylation by α-methyl-3,4-dihydroxy-DL-phenylalanine (α-methyl-dopa) have been extended. Inhibition by this agent of the formation of dopamine, tryptamine, tyramine, serotonin and phenylethylamine from their corresponding amino acids in man has been shown. That the decarboxylase step of serotonin synthesis is specifically attacked was demonstrated in two carcinoid patients by the observation of an elevated excretion of 5-hydroxytryptophan (5HTP) coincident with treatment. Administration of α-methyl-dopa to two patients with pheochromocytoma resulted in a slight (20%) but probably significant reduction in the excretion of catecholamine metabolites. Decarboxylase inhibition with α-methyl-dopa was shown pharmacologically in the dog by blockade of the inotropic response to L-Dopa and in man by decreased intestinal motility response to 5-HTP. Several additional inhibitors have been synthesized in the Merck, Sharpe and Dohme laboratories and are in various stages of animal and clinical evaluation.

Action and Metabolism of Drugs

In studies with eight different monoamine oxidase (MAO) inhibitors, we have observed an orthostatic hypotensive effect rather uniformly in

hypertensive subjects when evidence of effective MAO inhibition was demonstrable. The latter is indicated by increases in the urinary excretion of amines such as tryptamine and tyramine. Inhibitors containing the hydrazine moiety have frequently produced toxic effects in man at the doses required to lower blood pressure. Thus the preliminary finding that N-benzyl-N-methyl-2-propynyl-amine·HCl (MO-911) is an effective antihypertensive agent and potent MAO inhibitor in man is of special interest. Also, since the structure of this agent is different from that of previous inhibitors, the evidence of a causal relationship between MAO inhibition and blood pressure lowering seems fairly conclusive. Nonetheless, the exact mechanism is undetermined. Neuropharmacologic experiments in dogs and cats have shown a sympathetic ganglion blocking action of several inhibitors; however, this effect occurs only with large doses and is rather evanescent. Possibly only a slight modification of ganglionic function is sufficient to produce postural hypotension in man. Among other possibilities, peripheral and/or central accumulation of an amine such as dopamine could result in competitive block of norepinephrine action.

It is claimed that MAO inhibitors benefit patients with angina pectoris. A rational basis for this might be the observations in two patients receiving the inhibitor, isocarboxazid, of obtunded rises in pulse rate and blood pressure during exercise. This implies diminished cardiac response during exertion.

In 40 hypertensive patients treated for six weeks to fifteen months with α-methyl-dopa, initial sedation followed by subtle tranquilization and lowering of blood pressure which is predominantly orthostatic have been observed rather uniformly. The potency of the compound is enhanced in patients with impaired renal function and it is uniquely effective in emergency control of severe hypertension particularly if used intravenously. Recent availability of the L isomer (Aldomet) has largely solved the problem of high daily intake of capsules for maintenance therapy. Broad evaluation of this agent as an antihypertensive is currently in progress in 16 clinics. Only three of eight carcinoid patients have shown symptomatic relief with α-methyl-dopa. Development of hypotension has often prevented attainment of effective dose levels. However, decarboxylase inhibition appears to be a sound approach to treatment of carcinoid, in part because it now seems doubtful that it is a basis for development of hypotensive drugs. Biochemical studies in rats and guinea pigs (LCB), pharmacologic studies in dogs, and combined studies of drug metabolism and blood pressure responses in patients all suggest that α-methyl-dopa has an activity above and beyond decarboxylase inhibition. For the moment, it is assumed that a selective depletion of tissue stores of norepinephrine accounts for the lowering of blood pressure. Clinical studies of other

decarboxylase inhibitors, some with and others without norepinephrine-depleting properties, will be initiated shortly.

Miscellaneous

It is not established whether excess deposition of fibrin in clinical pathologic states is due to a deficiency of fibrinolysis or an excess of inhibitor activity. To explore this problem, a suitable method for measuring inhibitor activity has been developed.

Radioactive renography is being performed routinely in hypertensives in an attempt to evaluate its use in diagnosis of unilateral renovascular hypertension. To date, the only positives have been in four patients with proved renovascular lesions. Curative surgery was performed in these patients.

Section on Cardiodynamics

This Section has as its ultimate objective the study of the biophysical and physiologic behavior of the cardiopulmonary system of normal and diseased human subjects as they go about their usual daily activities and are subjected to various physiologic, psychic, pharmacologic, and other stressful interventions. The measurement of a large number of physiologic variables under conditions most nearly simulating normal activity has made the development of new methods of instrumentation essential. Progress in this broad area has and will continue to depend on a vigorous program in instrumentation development as well as a comprehensive study of the biomathematical models of the vascular and pulmonary systems. Thus, the major efforts of this Section have been directed along three lines: 1) instrumentation, 2) biophysics of the vascular system, 3) biophysics of the pulmonary system.

Instrumentation: The needs for improved instrumentation have made necessary a continuous instrument evaluation program. Most of the efforts in this area have been toward the development of a data processing and computing unit. Commercially available equipment has been modified to meet the biological and biophysical requirements of the Section. Many technical problems have been solved so that there is now available an efficient, smooth working, physiological recording system which permits the acquisition of multiple channels of physiological information from animal or man. These data may be recorded on conventional direct writing recorders, and at the same time stored by an electromagnetic FM tape system. Unique methods of programming the electromagnetic tape system to work either with an analogue to digital converter or

with an analogue computer have been worked out. For example, a given time increment of information, such as one heart beat, may be searched out and programmed to play repetitively for study and computation.

Pressure measurement has remained one of the most important unsolved problems, and therefore, new techniques are being sought. Several miniaturized pressure transducers have been investigated, one of which has shown considerable promise. However, none has been found sufficiently accurate for the more precise biophysical measurements. An extravascular pressure sensing device is being developed in conjunction with the Astra Corporation. This device can be surgically implanted in animals, and, it is hoped, will open the avenue to accurately telemetered blood pressure from living, intact, active animals. This information has not been available previously, and if successful, the attempt to measure it will be a major step toward the accomplishment of the primary objective.

The measurement of instantaneous blood flow in the living intact organism has remained one of the primary objectives. Studies in this area have been carried out in mechanical models and in the living animal. Experimental studies with a mechanical flow generating device have revealed that the relationship between the instantaneous pressure gradient in a cylindrical tube and the instantaneous pulsatile flow is such that it may be predicted mathematically with reasonable accuracy. The velocity profile was examined by the introduction of blue dye strands into the pulsatile flow and indicated that in the range of physiologic frequencies the velocity profile across the tube is blunt, with most of the fluid shear occurring at the boundaries of flow. The more peripheral sleeves of flow appear to be in phase with the pressure gradient, while the more central portions of the flow appear to be in quadrature with the pressure gradient. This finding is qualitatively in agreement with mathematical prediction.

In the animal, blood velocity has been compared from the simultaneous recordings from a Kolin electromagnetic flowmeter and the computed pressure gradient technique. Agreement of peak blood velocities by the two methods is quite good.

Cardiovascular Biophysics: The viscoelastic properties of the living vascular tissue have been studied. The results indicate that the pressure-diameter relationships of almost all blood vessels, in the systemic as well as pulmonary system appear to be described to a first approximation as a simple elastic system, particularly for the lower frequencies of pulsation. It would appear also that the longitudinal motion of blood vessels may be neglected as a boundary condition assumption in the solution of the hydrodynamic equations of flow.

The instantaneous blood velocity, pressure, pressure gradient and vessel radius are being measured and recorded on an electromagnetic tape system so that computation of the various terms of the Navier-Stokes equations may be accomplished. Preliminary evidence indicates that a large majority of these terms may be neglected. Therefore, it is reasonable that a relatively simple, yet realistic equation describing the pressure, flow, diameter relationships in the vascular system can be established.

In order to study the theoretical aspects of heart function, it is necessary to determine the nature of the load against which the heart must work. To this end, studies are being carried out to examine the input impedance of the pulmonary artery and the aorta. This is done by measuring simultaneously the instantaneous blood flow and pressure at the input of these two major vessels. These curves may be broken in to their respective Fourier series. Each term in the series may be represented by a complex number. The ratios of the corresponding harmonics of pressure and flow permit the computation of the complex impedance. The results to date indicate that the input impedance to the pulmonary artery consists of a very large real term and a relatively small imaginary term. Although the interpretation of this finding is somewhat involved, the major impedance to blood flow into the pulmonary artery seems to be related to vascular resistance. This is in contrast to the systemic side in which large reactive components of impedance appear.

Pulmonary Biophysics: Theoretical considerations have been developed which indicate that abnormal stress distribution within the structure of the lung may be the major factor in the production of the disruptive lesions of emphysema. The abnormal stress distribution could be the result of either congenital or acquired abnormalities of the smaller terminal air passages. Therefore, studies have been undertaken to examine the relationship of stress to strain in three dimensions in excised lungs. By using an improved intraesophageal pressure measuring system, the intrathoracic pressure is being measured simultaneously at three different sites along the esophagus. The difference between oral pressure and the intraesophageal pressures has been studied under static conditions in normal subjects. It has been possible to describe this relationship as a function of balloon volume, balloon position, and lung volume. The relationship seems to be independent of the rate of change of pressure or of the amplitude of pressure changes. This initial part of the project, which is of interest in itself, is preliminary to studying the pressure differences between the three balloons and the conditions of air flow, varying rates of respiration, and rapid respiratory maneuvers such as cough. If it can be demonstrated that there are significant differences in the stress distribution in the lung of normal individuals, as compared to those with chronic

bronchitis, evidence is gained for support of the theory that the disruptive lesions of pulmonary emphysema may be the result of abnormal physical stress.

New methods of studying the mechanical behavior of the lung have been developed. A unified three dimensional presentation of the three major variables controlling the lung behavior in health and disease has been developed in this laboratory and used to examine normal, cardiac, and emphysematous subjects. From these considerations a unique relationship between the maximum achievable expiratory flow and the degree of lung inflation has been discovered. Theoretical considerations of this unique flow-volume relationship have far reaching physical and physiological implications. Therefore, this relationship is being extensively studied in normal human beings. Theory would indicate that this relationship is controlled by the density and viscosity of the gas as well as the dimensions and physical properties of the intrathoracic airways. If it can be shown, experimentally, that the effect of either density or viscosity on this relationship was of minor importance, it would greatly simplify the mathematical description of this curve. Preliminary findings indicate that the viscosity of the gas breathed has very little effect on the maximum achievable expiratory flow at any given degree of lung inflation. This finding is in sharp contrast to the effect of gas density. If these preliminary findings are verified, it may be possible to establish a realistic and useful mathematical model of the lung from which the flow volume curve may be analyzed mathematically.

The investigative projects of the Section on Clinical and Experimental Surgery of the Surgery Branch have, as in past years, centered largely around methods for improving the surgical treatment of patients with congenital or acquired heart disease and in elucidating the physiologic factors which apply before, during and following the operative correction of such lesions. An important group of studies has continued to center around the clinical application of the artificial heart and lung machine. The artificial heart and lung machine itself has not been modified in the past year since it had been found to provide adequate support of the circulation for periods of up to two hours. It may be of interest that the machine has been accepted for commercial production.

Perhaps the most pressing clinical problem in the field of cardiovascular surgery is the surgical correction of mitral and aortic regurgitation. For the past two years, efforts in both the experimental laboratory and in the operating room have been directed at the perfection of a prosthesis suitable for total replacement of the mitral valve. Valves have been constructed with a dacron fiber base covered with a thin layer of polyurethane foam. They have been implanted into five patients with irretrievably damaged valves. Although no patient is presently living with a total valve replacement, the valve was in place in one patient for more than four months. Autopsy examination revealed that this valve was free of clot, covered with a thin layer of fibrin and had remained mobile. This result is sufficiently encouraging that further refinements in the design of the valve are being made in the hope that it will ultimately prove useful for routine clinical practice. An important part of the problem of prosthetic valve replacement, whether for the aortic or the mitral valve, is a choice of material. It is particularly difficult to evaluate materials when valves are constructed and placed in experimental animals since the technical aspects of the operation itself are so formidable. Accordingly, various fabrics such as dacron, silk, silastic, pericardium and nylon have been implanted into the wall of the right atrium in dogs. A study of the behavior of these materials in this location will undoubtedly indicate that certain fabrics are more desirable than others in valve construction.

In the prosthetic mitral valve which has been employed clinically, artificial chordae tendinae are necessary to prevent eversion of the valve leaflets from the ventricle into the atrium. An experimental study is underway to evaluate the ideal material for such artificial tendinae. Strands of various materials, such as stainless steel, silk, silk covered with plastic, etc., have been passed through the substance of the heart so that the fibers traverse the cavity of each ventricle. In addition,

various methods have been employed to fix the strands to each ventricular wall. Preliminary observations indicate that clotting is primarily related to the point at which the strand pierces the endocardium and that the substance of the strand may be of lesser importance. Fixation of the strands by mechanical means has been found inferior to direct suture.

In the clinical treatment of patients with various types of congenital and acquired heart disease principal efforts in the past year have been directed at surgery of the mitral and aortic valves. Continuing experience with the open surgical correction of calcific aortic stenosis, with followup catheterization information has revealed that in the majority of patients simple commissurotomy is not effective in relieving left ventricular outflow obstruction unless aortic regurgitation is produced. Accordingly, a portion of the aortic valve has been replaced with a prosthesis, ordinarily of woven teflon cloth, in an increasing number of patients. It soon became apparent that the prolonged period of aortotomy necessary in these operations could not be provided by coronary perfusion alone. For this reason selective hypothermia of the heart, induced by perfusion of the coronary vessels with refrigerated blood and a slush of ice and water around the left ventricle has been adopted. Utilizing this technique, safe periods of cardiac arrest up to two hours have been achieved. A study comparing the effects of cardiac hypothermia and coronary perfusion on ventricular function has been initiated.

One year ago two patients with functional hypertrophic subaortic stenosis were subjected to operation. It is impossible to resect the obstructing muscle mass and in these patients the lesion was treated by a vertical incision or myotomy across the contraction ring within the left ventricle. Although the initial hemodynamic results seemed encouraging, the procedure was not again used until these patients were studied one year later. At this time each was found to have virtually complete relief of the obstruction. Since increasing numbers of patients with this form of aortic stenosis are being seen, it is likely that this new operation will prove of increasing value.

Previous studies have indicated that it is advisable for every patient undergoing open heart surgery to be digitalized beforehand. There has been much conjecture as to whether or not extracorporeal circulation removes digitalis from the heart muscle and whether patients after open operations should receive additional digitalis. An experimental study was undertaken in which animals were digitalized and their digitalis level titrated before and after cardiopulmonary bypass. Preliminary work indicates that digitalis is fixed in heart muscle and no decrease in its effective level could be demonstrated after bypass. The work will be continued, more precisely, with the use of radioactive digitalis preparations.

It has been occasionally noted in patients undergoing prolonged cardiopulmonary bypass that oliguria or anuria occurs. Although a high level of free plasma hemoglobin is frequently present following long perfusions there has been no correlation between the occurrence of a high plasma hemoglobin level and the occurrence of renal complications. In an experimental study, p-aminohippurate and creatinine are being utilized to measure renal plasma flow and glomerular filtration rate in dogs in which the kidney is supported by an extracorporeal circulation. By means of this preparation the effects of pure hemoglobin and red cells debris on renal function may be evaluated.

In many clinics extracorporeal circulation is presently being used concurrently with deep hypothermia, heat exchange being accomplished in the extracorporeal circuit. It has been generally assumed that as the body temperature falls blood flow to muscle masses is decreased while that to the "vital organs" remains high or is increased. Experimental studies have been undertaken to measure the total blood flow and the vascular resistance of various circulatory beds during profound hypothermia. It has been a surprise to learn that at $10^{\circ}C$. the flow to the lower extremity is increased while that to the brain and splanchnic bed is lower than normal. Continuing investigations concerning the changes in hepatic blood flow under these circumstances are underway. When profound hypothermia is used, ventricular fibrillation is an almost invariable complication of the technique and in clinical practice large doses of quinidine and sometimes procaine amide have been given to prevent this arrhythmia. The direct myocardial effects of these agents were not known and, accordingly, left ventricular function curves were constructed in dogs in which quinidine had or had not been given. It was found that quinidine produced a marked depression of left ventricular function and a profound fall in mean arterial pressure indicative of peripheral vasodilatation. It would seem that the protective effects of quinidine are outweighed by these undesirable side effects.

Fifteen patients have now been studied in whom left ventricular outflow obstruction was found to be due to massive hypertrophy in the outflow tract of the left ventricle. An attempt to produce this lesion experimentally was made by constriction of the ascending aorta in puppies. These animals have now been followed for one year and all have been found to have massive hypertrophy of the outflow tract of the ventricle and in several a pressure gradient within the left ventricular cavity has been found at retrograde arterial catheterization. Although this experimentally produced lesion is probably not akin to the asymmetric hypertrophy most common in patients, it does provide an experimental tool by which operative treatment of this unusual form of aortic stenosis may be evaluated.

The complete anatomic correction of certain congenital lesions such as severe tetralogy of Fallot and true truncus arteriosus will necessitate replacement of the pulmonary artery. In a previous study, single pulmonary arteries were replaced with grafts of plastic material and this work has been extended to permit total replacement of the main pulmonary artery and its major branches. It has been found that the length of the rigid graft is of critical importance but that when this parameter is controlled, that dogs can survive for prolonged periods with such a prosthesis not containing a valve.

While the heart and peripheral circulation are physiologically separated, during cardiopulmonary bypass, a unique opportunity is afforded for studying the various direct myocardial and peripheral actions of many pharmacologic agents. The effects of various pressor amines and digitalis have been described and these studies suggested that a detailed investigation of certain anesthetic agents would be of value. In a study carried out in conjunction with the Department of Anesthesiology the anesthetic agent Halothane was introduced into the oxygenator for a period of five minutes. In six patients it was shown that there was immediate and progressive depression of myocardial contractility and a concomitant fall in systemic blood pressure indicating peripheral vasodilation. The effects of carbon dioxide and other gaseous agents on the heart and peripheral circulation can also be studied by this method and such projects have been initiated. The clinical observation was made that, following operations necessitating occlusion of the thoracic aorta, paradoxical hypertension following restoration of the circulation was far more frequent when Halothane anesthesia was employed. In an experimental preparation the aorta was occluded in dogs under either nitrous oxide-oxygen or Halothane anesthesia. Postoperative hypertension was uniformly noted when Halothane was employed. This action of Halothane may originate in an alteration of renal blood flow and this aspect of the problem is under investigation.

During the past year the Surgery Branch has supported a Resident in Orthopedic Surgery. Two investigative projects have been carried out in collaboration with the National Institute of Arthritis and Metabolic Diseases. There is no detailed knowledge concerning the pathogenesis of the bone changes secondary to cyanotic heart disease. Various experimental procedures have been employed to reduce arterial oxygen saturation and a series of animals in which cyanosis has been produced are being x-rayed at intervals to determine the progression of their pulmonary osteoarthropathy. Also a new lesion of the small blood vessels in joint tissue has been observed in a series of patients who had no symptoms of joint disease. The lesion can be characterized as an occlusive vascular one whose significance or etiology is entirely unknown. A detailed investigation of the incidence of this vascular lesion has been undertaken in an

effort to determine its possible relationship to the aging process.

Clinical studies in the Section of Cardiology during the past year have again focused on the applicability of Starling's law of the heart to man. A method was developed by which it is possible to determine directly the interrelationships between ventricular end-diastolic fiber length, diastolic tension, systolic tension and the rate of development of tension. These measurements may all be carried out by means of a specifically modified Walton-Brodie strain gauge arch sewn to the surface of the human heart. These studies have shown that as human myocardial fibers are progressively stretched, a progressive increase in diastolic tension, in the tension developed during systole, and in the rate of development of tension takes place. These observations are direct evidence of the operation of Starling's law of the heart in man. In other studies, on patients with mitral stenosis and atrial fibrillation, further evidence of the applicability of Starling's law was obtained. Continuous alterations of the length of a segment of left ventricular muscle were recorded at operation. On a beat-to-beat basis the length of a segment of left ventricular muscle at the end of diastole was found to correlate extremely well with the characteristics of ventricular contraction; the latter was assessed by a detailed analysis of individual left ventricular pressure pulses. Similarly, the left ventricular end-diastolic pressure appeared intimately related to the subsequent ventricular contraction in several patients with a closed chest studied at the time of left heart catheterization. Ventricular function or "modified Starling" curves were obtained in seven subjects with normal cardiovascular systems studied while under complete ganglionic blockade. "Effective" left ventricular end-diastolic pressures, cardiac output, and arterial pressure before and after the transfusion of 1500 ml. of blood were measured. In each instance ventricular filling pressure correlated well with the left ventricular stroke volume and stroke work, further supporting the concept that Starling's law of the heart applies to man.

Hemodynamic observations on the function of the left atrium were carried out on 50 patients with various forms of heart disease. It was found that in patients with left ventricular disease (arteriosclerosis, aortic valve disease, etc.) left atrial contraction elevated left ventricular end-diastolic pressure while maintaining the left atrial (and therefore the pulmonary capillary) pressure at a substantially lower level. This dissociation between left ventricular end-diastolic and mean left atrial pressures provides an "index" of left atrial function and emphasizes the clinical importance of left atrial contraction in patients with left ventricular hypertrophy. It was shown that, just as in the left ventricle, the pressure in the left atrium just prior to the onset of atrial contraction is an important determinant of the characteristics of atrial contraction.

Currently, measurements of two other basic parameters are being carried out. The left ventricular end-diastolic volume is being correlated with the left ventricular stroke volume and stroke work. Similarly, the rate of development of left ventricular pressure, i.e. the slope of the isometric pressure gradient, is being correlated with the end-diastolic segment length. These studies are designed to provide more detailed information about the hemodynamic determinants of the characteristics of ventricular contraction in man.

In a continuing investigation of the pharmacology of drugs which act primarily on the cardiovascular system it was shown that digitalis glycosides augment the contractile force of nonfailing human hearts. In these studies, carried out on patients at the time of "open heart" operations it was also observed that digitalis exerts a direct arteriolar constrictor effect. These studies may serve to change current concepts of "prophylactic digitalization." In other observations on digitalis glycosides it was demonstrated that the production of mild hyperthyroidism greatly augments digitalis requirements. It was shown that large doses of rauwolfia alkaloids abolish these increased digitalis requirements. Guanethidine, a drug which apparently releases tissue stores of catecholamines, was found to abolish many of the manifestations of triiodothyronine-induced hyperthyroidism. In other studies the effect of large doses of syrosingopine, a rauwolfia derivative, on the cardiac response to acute hypoxemia and exercise was evaluated. It was found that the tissue catecholamine depletion produced by this drug failed to alter the cardiovascular response to these two stimuli.

The development and evaluation of newer diagnostic technics was continued during the past year. In a critical appraisal of the Kr^{85} inhalation test for the detection of left-to-right circulatory shunts, in over 300 patients in whom the presence or absence of such a shunt was subsequently proved, it was shown that the Kr^{85} inhalation test probably represents the most accurate means for the clinical detection of circulatory shunts. A simplified technique for the detection of patent ductus arteriosus was developed. This consists of the injection of Kr^{85} into the thoracic aorta and measuring the appearance time in the expired air. This technique has been found particularly valuable in excluding a patent ductus arteriosus in patients prior to open heart operations. Both ascorbic acid and iced saline have also been employed as indicators in the study of patients with congenital heart disease. Ascorbic acid is detected by means of a platinum electrode, while temperature is sensed with a small thermistor. Both of these elements may be introduced directly into the arterial blood or into the heart. By these techniques indicator-dilution curves may be recorded without sampling blood and therefore they constitute an important advance in the use of indicator-dilution curves. Solutions of Kr^{85}

have also been employed as the indicator for the measurement of cardiac output by the indicator-dilution technic. Since Kr^{85} does not recirculate, inscription of the dilution curve is obviated, thus greatly simplifying the technic of cardiac output measurement. Transseptal left heart catheterization, developed in this laboratory two years ago, has been modified so that: 1) surgical exposure of the saphenous vein is avoided, 2) a smaller needle is employed for atrial puncture, 3) a larger catheter can be introduced into the left side of the heart, and 4) left heart angiography may be performed conveniently. This has greatly improved the clinical value of this technic.

Observations on 15 patients with idiopathic hypertrophic subaortic stenosis have been continued. This is the largest number of patients studied at any single hospital and detailed clinical, phonocardiographic, hemodynamic and angiographic observations have permitted a comprehensive description of this disease entity. In particular, a simple hemodynamic technic for the detection of this condition was described. This technic is based on an analysis of the response of the arterial pressure pulse to a premature ventricular contraction.

In studies performed in the experimental laboratory of the Section of Cardiology the effects of a variety of stimuli on the arteriolar and venous tone of the dog were examined. These investigations were carried out employing a preparation on total cardiopulmonary bypass in which the heart and lungs were excluded from the circulation. Thus, it was shown that veno-constriction accompanies arterial constriction when the pressure in the carotid sinuses and in the left side of the heart are lowered. Similarly, hypoxia and hypercapnia produce veno-constriction, but the increased arterial pressure which usually occurs with these stimuli results from an accompanying increase in the cardiac output. Hypothermia was shown to produce arterial and venous dilatation while hyperthermia has the opposite effect.

The mechanism of the inotropic, chronotropic and pressor effects of guanethidine was studied in normal dogs and in dogs following chronic cardiac denervation. The results of these experiments support the contention that guanethidine produces a positive inotropic and chronotropic effect by suddenly releasing the myocardial stores of catecholamines.

GERONTOLOGY BRANCH

The research program of the Gerontology Branch is directed toward (1) describing the biochemical, physiological and psychological changes that take place with increasing age in man and (2) investigating the basic biology of aging in order to understand age-dependent alterations in performance in man.

Physiological Studies

Longitudinal Studies: Age differences in biochemical, physiological and psychological characteristics of normal people still living successfully in the community are being evaluated. These subjects, ranging in age from 18 to 100 years, have agreed to return to the laboratory every 18 months for the remainder of their lives so that age changes can be recorded in individual subjects. Almost 200 subjects have received the first series of tests and 50 have been tested a second time. The program has been well received as evidenced by the fact that we are now making appointments with new recruits for December 1961. Due to limitations in space and staff, we can accept only two new subjects per week.

Because of the long term nature of this project, results on successive measurements cannot be analyzed as yet. However, members of this longitudinal sample have served as experimental subjects for many of the other studies on humans.

In collaboration with Dr. Bernice Cohen of the Johns Hopkins School of Hygiene and Public Health, the testing schedule has been expanded to include information on genetic background which should shed light on the question of the relation of assortive mating to longevity. Dietary habits of the subjects will also be surveyed in collaboration with the Maryland State Health Department and the Heart Disease Control Program of the USPHS (Dr. McGandy).

Renal Studies: Experiments have been conducted to determine whether the hemoglobin-haptoglobin complex is cleared from the plasma by the reticulo-endothelial system. Preliminary experiments indicate that Kupffer cells may be isolated from liver homogenates. If this method is successful, experiments will be performed using hemoglobin tagged with Fe^{59} to determine the presence of the hemoglobin-haptoglobin complex in isolated Kupffer cells in the dog.

No age differences in glomerular permeability were found using infusions of dextran of different molecular weights.

Studies of glomerular filtration of free hemoglobin (corrected for binding to haptoglobin) relative to inulin clearance have been carried out in 47 subjects aged 20-88 years. The ratio C_{Hb}/C_{In} averaged 0.055 (S. D. 0.019) and showed no significant correlation with age. Thus there is no evidence of a change in glomerular permeability with age. Tubular reabsorption of hemoglobin, calculated as the difference between filtered load and urinary excretion averaged 1.43 mg/min. (S.D. 0.96).

Measurements of creatinine clearance made over a period of 12 hours indicate similar rates of decline with age in both hospitalized and community residing groups.

Body Composition Studies: Estimates of lean body mass and body fat have been made utilizing the equations proposed by Keys and Siri which are based on body density and total body water determinations. In addition, a new equation was developed which introduces corrections for bone density (estimated from x-ray measurements of the phalanx) and for muscle mass (estimated from creatinine excretion). When fat content of the body was calculated from the Keys equation and expressed as a percentage of body weight an increase of 0.06% per year was found. However, when the new equation was used there was a decrease of 0.12% per year. The new equation gives a better estimate of body fat by introducing corrections for bone density and muscle mass, both of which show significant age decrements.

Energetics of Arm Motion: In cooperation with Dr. R. L. Ramsey, Medical College of Virginia, a description of the mechanical relationships of the human arm swinging maximally in alternating back and forth movements has been developed. During this maximum wagging exercise linear relationships were found between (1) period of swing and angular displacement (amplitude), (2) the impulse of the force applied to the limb and the log of the angular displacement and (3) action and angular displacement. The hypothesis that the slopes of these relationships were related to mechanical efficiency was not supported for any of them. Mechanical efficiency was calculated from the excess oxygen consumption associated with the work done. A family of curves was developed which related mechanical efficiency to age at different amplitudes of swing. Young subjects (age 30) showed an increase in efficiency with increasing amplitude of swing, whereas in old subjects (age 70) efficiency did not increase with increasing amplitudes of swing. In both old and young subjects, the angular proportion of the swing during which force was exerted by the muscle decreased (93% to 57%) with increase in amplitude (0.36 Rad to 1.85 Rad).

The delineation of the mechanics of arm movement provides a basis for understanding age changes in the ability of an individual to

perform simple tasks efficiently. These measurements may provide a simple objective test of muscular efficiency that can serve as one factor in an index of aging.

Cardiovascular Physiology: Beginning November 1, 1960, Dr. William Milnor became Assistant Chief of the Gerontology Branch. Under his direction, clinical research programs of the Branch will be expanded with particular reference to cardiovascular and pulmonary physiology. In addition, animals will be used for the study of pulmonary vascular elasticity.

Psychological Studies

Investigators in the Psychology Section provide information on the psychological performance and personality characteristics of subjects included in the longitudinal study of aging. In addition to standard tests of intellectual performance, standard questionnaires to evaluate personality characteristics are being administered. Estimates of reaction time, α - frequency of the EEG, spinal reflex amplitude, heart rate variability and motor time in these subjects are also being made.

A substantial correlation (0.81 with $p < 0.01$) between mean reaction time and mean alpha wave frequency of the electroencephalogram has been observed in a group of 13 subjects. Correlation between these variables within individuals varied over a wide range of values (+0.03 to + 0.65). When the effects of several other variables related to reaction time were partialed out by means of multiple regression analyses, the low individual correlation of 0.03 between reaction time and alpha wave frequency was increased to 0.29. The variables partialed out were amplitude of spinal reflex, heart rate and motor time (the time which elapses between the appearance of a muscle action potential and the mechanical response of the muscle). These variables are regarded as indices of the activity level of the brain stem reticular system. These measures also account for 1/3 of the total variance of the speed of response. Hence, it may be inferred that speed of response is influenced by general excitatory states of activity in the brain and spinal cord which may fluctuate from moment to moment.

The effect of interpolated activities on short term memory was tested in subjects with a high level of educational attainment. In contrast to the previous study which showed greater interference among old than young subjects with a low level of education, age differences in this group were not statistically significant. However, when the task was shifted to the judgment of a time interval (length of time a stimulus light was on), the older subjects showed a significantly greater interference effect. In

this experiment the young subjects were more effective than the old in learning the original stimulus series so that a covariance analysis was required to test the significance of the age difference in re-learning scores. This analysis showed that the age difference was statistically significant. It is inferred that external cues were available in the experiment requiring judgments of size which were not available in the experiment involving time judgments and that the group of more capable subjects used these cues to a greater extent than did the subjects of lower capabilities.

Basic Biology

Cellular and Comparative Physiology: The general research objectives and activities of the Cellular and Comparative Physiology Section continue in a twofold direction: (1) the description of cellular and organismic changes in humans and appropriate experimental animals during aging and (2) the measurement of the effects of alterations in the environment on the performance and mortality of experimental animals.

Histochemical methods for the localization of reductases in tissue cultures of developing embryonic muscle tissue have made possible the demonstration that succinic dehydrogenase, DPN · H - cytochrome c reductase and TPN · H - cytochrome c reductase increase dramatically concomitantly with multinuclearity. This finding indicates an increase in mitochondria following cell fusion.

In order to determine whether the multinuclear cell becomes dependent on oxidative pathways exclusively, cultures were grown in the presence of antimycin A. The results indicate that although the mononucleated cell can maintain itself and proliferate in the presence of the blocking agent, the multinucleated cells are selectively killed. Thus muscle differentiation may depend upon an obligatory aerobic metabolic process. These studies lend support to the hypothesis that growth (cell proliferation) and differentiation are mutually exclusive processes.

Histochemical localization of reduced nucleotide reductases has also been useful in demonstrating cross-striations in developing muscle tissue in tissue cultures. These striations can be detected since the mitochondria are oriented along the A bands.

The recording spectrophotometer has been modified to permit continuous scanning of the absorption spectrum of individual cellular elements. By using appropriate corrections, the standard error of replicate readings has been reduced to 1-3% of the mean value.

Using this method, measurements of ploidy variability in developing chick muscle cultures have been made to compare the distributions in mononuclear and multinuclear cells. Preliminary results show a unimodal distribution of DNA values for the muscle cell nuclei which corresponds to the lower peak (diploid) of the bimodal distribution found in mononuclear cells. If this result is confirmed, it will offer evidence that muscle is a true syncytium. This technique will make it possible to determine whether the nuclei of the multinuclear muscle cells are, in fact, nonproliferative. Measurements of DNA per cell will be extended to comparisons of cells from young and old animals to test the hypothesis that the frequency of mitotic "accidents" increases with age.

Muscle cells obtained from biopsy material taken from adult and aged humans can be maintained in tissue culture. Cells originating from mature skeletal muscle tissue are capable of neoformation of multinuclear muscle cells. After 2-3 weeks of culture, long multinuclear ribbons form in the outgrowth area of the original explants. These resemble the early multinuclear cells of chick embryonic muscle and have a highly refractile cytoplasm which, after fixation and staining, shows many longitudinal fibrils. Removal of the original explants prior to multinuclear cell formation did not affect the process. This indicates that the multinuclear cells do not arise simply by the "budding" of injured mature muscle cells. Efforts are being made to isolate the various cell types found in mature muscle in order to determine the source of the myogenic elements.

Further studies have been made of the age pigments which accumulate in the human myocardium. The particles have the following average dimensions: major axis, 1.39 ± 0.06 micra; minor axis, 0.97 ± 0.04 micra. Specific gravity of the particle is 1.18 ± 0.03. Measurements of reduced vs. oxidized difference spectra indicate that isolated age pigment particles contain very little flavoprotein, cytochrome or cytochrome c reductase which are present in mitochondria. Tests for some of the Krebs cycle oxidases have also been negative. Cathepsin and acid phosphatase activities were pronounced and there were traces of acid deoxyribonuclease and ribonuclease in the particles. Thus the enzymatic behavior of the age pigment particles resembles that of lysozymes more than mitochondria.

Alkaline hydrolysis of lipids extracted from age pigment does not greatly alter the fluorescence. Gas chromatography of fluorescent fractions separated on silicic acid columns and subjected to methanolysis has revealed a preponderance of unsaturated over saturated fatty acid esters. The yellow fluorescent material also shows an infrared spectrum very similar to that of old oxidized samples of animal cephalin.

Data have been obtained that indicate that the photoreduction of TPN (in spinach chloroplasts) does not require the utilization of high energy phosphate bond energy (ATP). The firefly assay system was adapted for the continuous measurement of ATP levels in illuminated chloroplast suspensions.

Studies of the effects of environmental factors on the longevity of Drosophila melanogaster have been continued. Flies reared in environments free from micro-organisms live slightly longer than flies reared in a non-sterile environment. Exposure to 50,000 R of radiation increases (by 50%) the longevity of flies in non-sterile environments, but reduces longevity by about 20% in flies reared under sterile conditions. When flies are reared in an environment of 100% oxygen, the rate of aging is increased by about 50%, whereas, at lowered oxygen tensions (1-2% O_2), the rate of aging is reduced by about 35%.

Extensive time lapse records of the development, function, senescence and death of a number of species of coelenterates have been made, The individual species of this phylum have widely different longevities, ranging from a few days to many years. Stolonic fusion, hydranth regression, digestion, respiratory movements, growth, cleavage, and other functions have been recorded. Senescence and death of individual hydranths of Campanularia begins with a gradual contraction of the tentacles and is followed shortly by lysis and contraction of the hydranth with the return of its contents to the colony.

Nutritional Biochemistry

Three general areas have been investigated during the past year within the Section on Nutritional Biochemistry. These included studies on (1) age differences in tissue metabolism of the rat, (2) biochemical and physiological differences between normal rats and those whose life span may be increased through dietary restriction and (3) the effect of diets with respect to their potential to increase life span.

Among these studies has been an investigation on the effect of age on protein catabolism. Previous studies have indicated a high catheptic activity in the livers and kidneys of senescent rats. Since the in vitro determination of the enzymatic activity measures the breakdown of protein, a higher rate of catabolism of tissue proteins in old animals may be inferred. The earlier study (age differences in enzymatic activities of tissues during protein depletion and repletion) may be criticized on the grounds that it did not measure protein metabolism in normal animals. Therefore, the rate of protein catabolism was estimated by injecting radioactive methionine into animals of different ages and measuring the rate of disappearance

of S^{35} from the soluble protein fractions of various tissues. Although the rate of loss of radioactivity in the soluble protein fraction of liver tended to be higher in the older animals, statistical significance could not be established in this tissue nor in kidney, heart or muscle. Thus these studies again have failed to demonstrate any effect of age on protein metabolism.

Mitochondria from liver and kidney tissues of old and young rats have been separated. In both tissues succinoxidase activity per unit of washed mitochondria was the same for both old and young rats. The evidence thus suggests a senescent loss of whole mitochondria rather than a gradual loss of enzyme per mitochondrion. In these experiments, protein nitrogen content and turbidity of washed suspensions were used to estimate the number of mitochondria in the suspensions. The use of millipore filters for separating mitochondria is being explored to facilitate actual counting of the mitochondria. Studies are also being conducted to produce suspensions of isolated cells from other tissues such as the central nervous system for use in metabolic studies. Preliminary experiments show that incubation of minced cerebellum with papain and versene yields a suspension containing free Purkinje cells which can probably be isolated for metabolic studies and the preparation of mitochondria.

In past experiments, the age differences demonstrated in the concentrations of various enzymes in the kidney tissue of the rat have been relatively small (10%) in comparison to the large decrements in renal function observed in human subjects (60%). It is possible that aging affects the kidneys of these two species at different rates or that the enzymes chosen for investigation were not sensitive indices of renal function. Therefore, attempts were made to determine the effect of age on renal tubular transport in an in vitro system by measuring the accumulation of PAH by renal cortical slices from rats. Tubular function was estimated by the ratio of the concentration of PAH in the slice to that in the medium (S/M). The results demonstrated a tendency toward slightly lower S/M values in the senescent animals, but even this decrement was explained by a decrease in the number of cells as measured by the concentration of DNA in the tissue samples.

In another study, an estimate of age differences in total renal capacity was measured by the amount of renal tissue produced following unilateral nephrectomy in the rat. In addition to the total amount of tissue regenerated, the concentrations of succinoxidase, alkaline phosphatase, DNA, RNA and protein nitrogen were also determined. Although a greater degree of hypertrophy was found in the young animals (44%) as compared to senescent ones (33%), the concentrations of the various tissue components (DNA, RNA, protein N and enzymes) per unit wet weight in the

hypertrophied kidneys did not vary by more than 10% from values found in the normal kidneys and no marked age differences were observed. At the present time, it is not possible to attribute the observed differences in the degree of hypertrophy specifically to aging since the older animals, as indicated by body weight changes, did not seem to recover from the operation as well as the young.

In view of the fact that the same tests have not been carried out in the rat as in man, clearance techniques for the estimation of renal function in the rat are being developed. At present it is possible to measure renal clearances in unanesthetized rats under conditions in which inulin and PAH plasma levels remain constant for thirty minutes and the bladder is adequately rinsed to assure complete urine collecting. In future experiments, it will be possible to compare the effect of age in man and the rat by the same renal function tests.

For many years, it has been well known that the life span of the rat and mouse can be increased by severe dietary restriction. In order to understand the mechanism of this phenomenon, experiments were carried out to determine the biochemical and physiological differences between normal animals and those subjected to such dietary restriction. The results of this experiment demonstrated increased concentrations of succinoxidase in liver tissue and alkaline phosphatase in kidney tissue of the restricted animals. Since the previous study had indicated that the concentrations of these enzymes are lower in younger animals, these data do not support the concept that the restricted animal is similar to a chronologically younger animal. Similarly, the activity patterns measured in both suspension and wheel type cages also fail to support the hypothesis that the restricted animal is similar to a chronologically younger animal. Although dietary restriction has been shown to increase the life span in rats and mice, the application of this principle to species exposed to everyday stress is questioned in view of the work of McCay showing that the restricted dog succumbs much more readily to bacterial infection and parasitic infestation than does the normal animal. Since our experiments demonstrated that alterations in the concentrations of certain tissue enzymes do occur during dietary restriction, it may be possible to choose a diet which will support normal growth and still provide the necessary enzymatic alterations in the tissues to increase life span. Our first experiment in this direction demonstrated that it is possible to increase the concentrations of selected enzymes in various tissues by feeding growing animals diets which will promote optimum growth but which differ only in the quality (casein vs. whole dessicated liver) or quantity (20%-32% protein) of the dietary protein. Thus far, these experiments have not been totally successful since the enzymes affected by this means were not identical to those altered by dietary restriction.

Section on Intermediary Metabolism

The research activities of this section are concerned with the mechanisms of oxidative phosphorylation associated with the respiratory chain and with the substrate level reaction associated with the α-keto-glutarate oxidation.

The chemical reactions associated with the synthesis of ATP during oxidations in the mitochondrial electron transport chain are not known. The work carried out clearly implicates a dithiol grouping in the process. The evidence rests on the observations that arsenite, under special conditions, uncouples oxidative phosphorylation in liver mitochondria, exposes an ATPase and inhibits the exchange of P^{32}-phosphate with ATP. The effects are quite analogous to those of 2, 4-dinitrophenol. The involvement of dithiols in respiratory oxidative phosphorylation brings out the similarity of the process to the "substrate level" phosphorylation in the oxidation of α-ketoglutarate where the primary energy-trapping reaction involves the reduction of the disulfide group of lipoic acid and simultaneous formation of a thiol ester. A related mechanism, involving the oxidation of a charge-transfer complex between a disulfide and a reduced electron carrier to the corresponding high-energy thiol derivative, has been proposed to explain the role of dithiols in the respiratory phosphorylation system.

The mechanism proposed above is partly based on the results obtained in an investigation of the mechanism of the dihydrolipoyl dehydrogenase reaction. This enzyme has been identified as a flavoprotein, and it carries out the terminal reduction of DPN by dihydrolipoate in the sequence of reactions resulting in the oxidative decarboxylation of α-keto-glutarate. Based on the inhibition of the enzyme by arsenite and cadmium ions, the involvement of a second oxidation-reduction "prosthetic" group, namely, a disulfide-dithiol, has been identified. An interaction between the flavin-adeninedinucleotide and the dithiol results in the appearance of an absorption maximum at 530 mμ in the reduced enzyme. A model for this interaction has been discovered in mixtures of reduced lipoate and flavinmononucleotide (FMN). In the absence of any enzyme, the two compounds form a dissociable molecular complex with an absorption maximum at the same position. On standing, the complex breaks down to the lipoic disulfide and $FMNH_2$. Neither the model complex nor the reduced enzyme show evidence for free radicals in the electron spin resonance spectrum. The complex formed from reduced lipoate and FMN is analogous to the complex proposed in the scheme for respiratory oxidative phosphorylation. Preliminary evidence suggests that the primary oxidation event in α-keto acid oxidation may involve an unknown grouping which comes into effect even before lipoate is reduced.

The dynamic turnover of mitochondria in rat livers has been studied. S^{35}-methionine and C^{14}-acetate were injected in the animals and the rate of loss of label followed in four mitochondrial components (soluble protein, insoluble protein, lipid and cytochrome c). The results showed that all components had essentially the same half-life of 10.3 days which favors the conclusion that mitochondria are synthesized and broken down as units. The individual constituents do not appear to be replaced after the synthesis of the particle. No difference in turnover rates has been detected in senescent (20-22 month) compared to adult (12 month) rats.

Section on Biophysics

The research program of this section is directed toward an understanding of cellular mechanisms and their relationship to aging. At present attention is being directed toward cellular processes in cultures of the protozoan Euglena which can be artificially "aged" and to the relation between structure and function in muscle enzymes with special reference to the role of -SH groups.

Experiments on the effect of steroid hormones on cultures of the protozoan Euglena have revealed items of importance. First, the increase in respiration caused by minute quantities of testosterone is noticeable only in the actual presence of the hormone, and does not appear to persist after the cells are washed. Second, the effect of testosterone is not evident unless a source of nitrogen is in the medium. It thus finally appears possible to make an hypothesis on a biochemical or biophysical level – e.g., that testosterone controls membrane permeability. This hypothesis is further suggested by the finding that vitamin D, also a steroid, enhances the growth of these cells in a low Ca^{++} medium, and work has now begun on the transport of Ca^{++} into these cells in the presence and absence of added vitamin D.

The effects of age and starvation on these protozoan cultures have also been examined in a preliminary fashion. Viable cells have been maintained for many months in the absence of either a carbon or a nitrogen source. Preliminary indications were that drastic changes occurred in both the RNA and DNA content of the cells. Prolonged checking of the analytical procedures for DNA analysis has shown that the DNA analyses were not reliable and a series of minor modifications has been worked out which will now permit proper measurement of the DNA content at various times of "aging." The RNA content decreases from about 30 μg/million cells to about 5 μg/million cells or less. During the lag period that occurs when the cells are put into complete medium the RNA is resynthesized. The length of the lag has been found to increase with the

time during which the culture was "aged" and some quantitative data on the relation between time of aging and length of lag have been obtained. When the cells are "aged" in a medium that contains no sulfur it is found that they cannot survive for more than about one month. Thus the mechanisms which permit the cell to survive in the face of nitrogen or carbon depletion do not function for the case of sulfur depletion. The existence of these adaptive regulatory mechanisms suggests that experiments on the content of certain enzymes in these cells after various times of aging would be the most profitable direction for this work to proceed.

The study of the role of -SH groups in muscle proteins has provided several new insights on the nature of the enzymatic process in myosin. Earlier in this project it had been shown that Cu^{++}, Cd^{++}, and Zn^{++}, like parachloromercuribenzoate (PCMB), would enhance the ATPase activity of myosin if these reagents were present in low concentrations at high temperatures (e.g., 25°C). At high concentrations, the ATPase activity was inhibited, and at low temperatures inhibition occurred at any concentration of reagent. For ITPase activity only inhibition was observed. We have now studied the rates at which these reagents interact with myosin and the way in which the presence of the substrate – either ATP or ITP – affects this rate. From these studies it appears that there are at least two -SH groups on the active site. One -SH group is involved in the ability of myosin to differentiate between ATP and ITP, and may be involved in a small conformation change at the site. Reaction of the second -SH group with any of these reagents leads only to inhibition. Studies on the tryptic digestion of myosin have shown that the active site is located on a trypsin resistant part of the myosin molecule and that the site does not interact appreciably with other parts of the myosin molecule. Several differences between Cu^{++} and PCMB have also been found. In particular, the use of Cu^{++} at several pH values has revealed the presence of an ionizing group, with an apparent pK near 7.4, that influences the enzymatic activity. The results of this work on myosin suggested that the adenine part of the DPN coenzyme molecule might interact with some dehydrogenase enzymes in a way similar to the interaction of the adenine part of ATP with myosin. Experiments with lactic dehydrogenase were begun with these hypotheses in mind. We have found a certain similarity between the effects of Cu^{++}, Ni^{++}, Cd^{++}, Co^{++}, and Zn^{++} on these two enzymes, and, in addition, have succeeded in demonstrating that there is an -SH group on lactic dehydrogenase, the access to which is controlled not by the adenine but by the phosphate group on DPN which is closest to the nicotinamide portion of this coenzyme. Further work is in progress.

Section on Molecular Biology

The principal concern of this section in the past has been the function of metal ions in biological processes. During the last year, however, the focus of attention has been an artificial reaction of metals with biological molecules in order to elucidate their structure.

Structure of Nucleic Acids. It is believed at the present time that all of the hereditary information that brings about the development of an organism as well as its degradative changes on aging is carried in molecules of deoxyribonucleic acid (DNA). The material consists of four basic building blocks, adenine, cytosine, guanine, and thymine, of which hundreds are joined together in a chain by means of ribose and phosphate units. The information code is believed to consist of the sequence in which these building blocks are arranged; such sequence determination is therefore of utmost importance to an understanding of the hereditary code. Attempts to determine this sequence have, up to this time, been confined to enzymatic degradations. Unfortunately, however, no enzymes are available for splitting specific bonds in nucleic acids. The present effort is to develop a chemical reaction capable of such specificity.

Complexes of salicylaldehyde with copper, nickel, and cobalt have been found to react with adenine to produce substances that are different and more stable than the substances produced from cytosine, guanine, and thymine. Using various combinations of metals, salicylaldehyde, and the four bases, their ribosides and their riboside phosphates, it is indeed possible to distinguish all of these substances qualitatively and some of them quantitatively by simple color tests. So far, this differentiation has been possible only for the molecules that have been disassembled from the chain. The metals have been found to react also with ribose, making the differentiation on the chain more difficult, but various techniques are being used to overcome this difficulty.

Structure of Hemoproteins. Meanwhile, studies on the relation of rotatory dispersion of the hemoproteins to their structure have continued. Hemoproteins are proteins containing an iron complex, heme; the hemoproteins catalase and hemoglobin contain four hemes per molecule, whereas peroxidase and myoglobin contain only one heme per molecule. The rotatory dispersion is a plot of optical activity vs. wave length; this plot exhibits a "Cotton effect," i. e., an S-shaped curve, in the visible when the heme group is asymmetrically placed, but the plot is essentially parabolic when the heme is not asymmetric. All four of the molecules, catalase, peroxidase, hemoglobin and myoglobin, exhibit the Cotton effect when the molecules are intact, and they lose the effect when the natural folding of the protein molecule is changed in some manner into a more irregular shape.

In the case of catalase and hemoglobin, these changes were shown to be accompanied by splitting of the 4-heme molecules into 1-heme quarters or 2-heme halves; here the phenomenon can be explained if each heme is symmetrically surrounded by protein and the presence of additional hemes in the molecule destroys this symmetry. However, in the case of peroxidase and myoglobin, the molecules cannot be broken down, and the rotatory dispersion changes can be explained only if one accepts the following generalization: The Cotton effect associated with the heme absorption is a measure of the ordered arrangement of the protein and disappears when the structure of the protein has been destroyed.

This generalization has been applied in a study of the stability of the heme-protein site in catalase. The heme was removed and then added back to the protein. Four molecules of heme still reacted with each molecule of the catalase protein, showing that the site on the protein, to which the heme had been attached, was not destroyed in this procedure. However, the newly reconstituted hemoprotein showed no Cotton effect; hence the structure of the protein as a whole has been destroyed. Therefore, the site for heme attachment remains intact even when the gross structure of the protein is destroyed.

Metal Ions in Enzymatic Reactions. In the past we have studied the effect of metal ions on the substrates of enzymatic reactions. This year attention has been directed to the interaction of metals with proteins, specifically bovine plasma albumin (BPA). The most striking observation has been that the BPA molecule dimerizes in the presence of copper, but is not affected in this way by any other metal. Also, copper and cobalt shift the equilibrium between two forms of BPA, although manganese has no effect. A possible explanation of the specificity of manganese in many enzymatic reactions is that manganese, unlike other metals, will not change the structure of the protein.

One of the most puzzling aspects of the activation of enzymes by metal ions has been the fact that the order of effectiveness of the various metals differs from enzyme to enzyme, and is generally different from the order of effectiveness of metals in non-enzymatic catalysis. An examination of enzymatic reactions has shown that these discrepancies can be explained by the fact that the order may be the same in enzymatic as well as non-enzymatic reactions, but this same order applies in enzyme inhibition. Thus the effectiveness of a metal in any enzymatic reaction depends upon a compromise between ability to catalyze the reaction and yet to be minimally destructive to the enzyme.

Serial No. NHI-1
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Mathematical Models of the Cardiovascular System.

Principal Investigator: Donald L. Fry, M.D.

Other Investigators: Samuel M. Fox III, M.D., Dali J. Patel, M.D., Joseph C. Greenfield, Jr., M.D.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2
Professional: 2
Other: 0

Project Description:

Objectives: To establish practical yet realistic mathematical descriptions of the pressure versus flow relationship in the living vascular bed.

Methods Employed: The pressure and flow relationships are under study in mechanical models as well as the living vascular tree. The methods used permit the continuous measurement of instantaneous flow, pressure, and certain vascular dimensions.

Major Findings: Experimental studies with the mechanical flow generator have revealed that the relationship between the instantaneous pressure gradient in a cylindrical tube and the instantaneous pulsatile flow bear relationships to one another which may be predicted mathematically with reasonable accuracy. The velocity profile examined by the introduction of blue dye strands into the pulsatile flow indicate that in the range of physiological frequencies the velocity profile across the tube is blunt with most of the fluid shear occurring at the boundaries of the flow. The more peripheral shells of flow appear to be in phase with the pressure gradient while the more central portions of the flow appear to be in quadrature with the pressure gradient. This is qualitatively in agreement with mathematical prediction. Studies are in progress to quantify this observation with high speed motion pictures.

Preliminary data on the electrical recording calipers used to measure strain in the vascular system have shown a good dynamic response and a low mechanical impedance. Several studies have been

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Mathematical Models of the Cardiovascular System.

finished and many are in progress evaluating the visco-elastic properties of the vascular bed with this device. Most of the findings to date in the foregoing study have been published or reviewed in the publications listed under Part B.

Significance to bio-medical research and the program of the Institute: The findings of this broad program are to be used in support of the more clinical studies of cardiovascular dynamics and pulmonary mechanics being carried on in this section.

Proposed course of project: To further develop and establish the validity of the mathematical model of the cardiovascular system so that inferences regarding the physical properties, structure and function of the intact vascular system may be drawn from relatively simple and accessible peripheral information such as the subcutaneous vascular pulsations etc.

Part B included	Yes	No
	x	

Serial No. NHI-1

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Individual Project Report
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Fry, Donald L., M.D. The Measurement of Pulsatile Blood Flow by the Computed Pressure Gradient Technique. IRE Transactions on Medical Electronics. ME-6, 259-264, 1959.

2. Fry, Donald L., M.D. Certain Aspects of Hydrodynamics as Applied to the Living Cardiovascular System. IRE Transactions on Medical Electronics. ME-6, 252-259, 1959.

3. Fry, Donald L., M.D. Panel Discussion of Methods of Flow Estimation by Pressure Sensing Techniques. IRE Transactions on Medical Electronics. ME-6, 264-266, 1959.

4. Fry, Donald L., M.D. Physiologic Recording by Modern Instruments with Particular Reference to Pressure Recording. Physiol.Rev. October 1960.

5. Fry, Donald L., M.D., Noble, F.W. and Mallos, A.J.,B.S. An Electric Device for Instantaneous and Continuous Computation of Aortic Blood Velocity. Circ. Research 5:75-78, 1957.

6. Fry, Donald L., M.D., Mallos, A.J., B.S. and Casper, A.G.T.,B.A. A Catheter Tip Method for Measurement of the Instantaneous Aortic Blood Velocity. Circ. Research 4: 627-632, 1956.

Serial No. NHI-2
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Influence of Acute Coronary Ligation on the Left Ventricular Ejection Velocity in the Dog

Principal Investigator: Guy O. Barnett, M.D.

Other Investigators: Alexander Mallos, B.S., Samuel M. Fox III, M.D.
Donald L. Fry, M.D.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 0.5
Professional: 0.5
Other:

Project Description:

Objective: The method of velocity approximation of Fry and Mallos was used to give further insight into the hemodynamic changes following acute coronary artery ligation. This will be used as background information for the further study of the hemodynamic changes following the production of chronic coronary insufficiency in the dog.

Methods Employed: In the thoracotomized, anesthetized dog, acute coronary insufficiency was produced by transient ligation of the left anterior descending coronary artery. The instantaneous aortic velocity was measured through a double-lumen catheter placed in the ascending aorta. Left ventricular and central aortic pressures were measured with catheter manometer systems. "Myocardial contractile force" was measured by the strain gauge arch method of Brodie. An epicardial EKG was obtained from the area of myocardium made ischemic by the ligation.

Major Findings: Following acute coronary artery ligation, there were significant changes in the contour of the ejection velocity before any change occurred in left ventricular and diastolic pressure, aortic pressure, or in the standard electrocardiographic leads. These changes were reversible only if the ligation was removed within 1-2 minutes. This material was presented at the 32nd Scientific Session of the American Heart Association in Philadelphia in October 1959.

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Serial No. NHI-2

PHS-NIH
Individual Project Report
Calendar Year 1960

Influence of Acute Coronary Ligation on the Left Ventricular Ejection Velocity in the Dog

Significance to bio-medical research and the program of the Institute: There is a need of greater understanding of mechanism and possible mode of treatment of acute coronary insufficiency. These studies are basic to further understanding.

Proposed course of project: This project has been concluded and a paper is in preparation.

Part B included	Yes	No
		x

Serial No. NHI-3
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Measurement of Relationship of Diameter to Pressure in the Aorta.

Principal Investigator: Guy O. Barnett, M.D.

Other Investigators: Alexander Mallos, B.S., and Allan Shapiro

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1
Professional: 0.5
Other: 0.5

Project Description:

Objectives: In order to calculate the instantaneous aortic flow from the instantaneous velocity, it is necessary to evaluate the instantaneous aortic diameter (flow = velocity x diameter).

Methods Employed: In the anesthetized thoracotomized dog, the instantaneous aortic diameter was obtained using strain gauge calipers specially devised by Mr. Mallos. The aortic pressure was monitored by a catheter manometer system. Data were gathered during periods when the pressure was changed both by changing the blood volume and by the administration of nor-epinephrine.

Major Findings: The experimental data showed a linear relationship between mean diameter and mean pressure - a relationship which was not changed by the administration of nor-epinephrine. The relationship of diameter to pressure during each cardiac cycle was also evaluated. It would appear that the use of the mean systolic diameter would be satisfactory in the computation of flow.

Significance to bio-medical research and the program of the Institute: The establishment of the relationship between vascular diameter and intravascular pressure will greatly simplify the computation of instantaneous blood flow from blood velocity measurements.

Part B included:	Yes	No
	X	

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Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Barnett, Guy Octo, M.D., Mallos, Alexander J.,B.S., and Shapiro, Allan. The Relationship of Aortic Pressure and Diameter in the Dog. J. of Appl. Physiol. IN PRESS.

Serial No. NHI-4
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in Pulmonary Circulation

Principal Investigator: Dali J. Patel, M.D., Ph.D.

Other Investigators: Donald L. Fry, M.D.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1
Professional: 0.5
Other: 0.5

Project Description:
Objectives: To analyze the characteristics of the pulmonary vascular bed to find an acceptable mathematical model for the system.
Major Findings: A study of the pressure-diameter relationship in the pulmonary artery in dog, is already completed. The input impedance of the pulmonary artery in dog is being computed at present. This is done from simultaneously obtained pressure and flow curves. These curves are broken down by Fourier analysis (using digital and analogue methods) and the impedance is computed for each term of the Fourier series. This impedance is further broken down into the real and imaginary parts. Attempts are made to test the simplest electrical analogues with these results.
Significance to bio-medical research and the program of the Institute: These findings may be used to more accurately define the hydrodynamic load that the heart must support.
Proposed course of project: This project will be continued with similar studies being undertaken at Mt. Alto Veterans Hospital in collaboration with Dr. Peter Luchsinger.

Part B included	Yes	No
	x	

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Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Patel, Dali J.,M.D., Schilder, Donald P.,M.D., Mallos, Alexander, J., B.S. Mechanical Properties and Dimensions of the Major Pulmonary Arteries. J. of Appl. Physiol.15:92-96, 1960.

Serial No. NHI-5
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Length Changes in the Aorta.

Principal Investigator: Dali J. Patel, M.D., Ph.D.

Other Investigators: Donald L. Fry, M.D.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.5
Professional: 1
Other: .5

Project Description:

Objectives: The distensibility characteristics of dog's aorta with simultaneous measurements of length, pressure and diameter are studied.

Methods Employed: Anatomical models of the aorta with its major branches at various pressures are made. The characteristic impedance of the aorta is being evaluated as well as the input impedances of various major vessels.

Major Findings: The relationship between longitudinal strain, circumferential strain, lateral intravascular pressure, blood velocity, and longitudinal motion was examined in the aortae of twenty-five living thoracotomized dogs. Electrical calipers with adequate recording characteristics were used for instantaneous measurement of diameter and length. Results indicate: (1) With inspiration the thoracic aorta increased in length and the abdominal aorta shortened. (2) During a cardiac systole the length in the thoracic aorta increased with a rise in pressure; mean value for longitudinal extensibility x 10^3 was 15 ($\pm$ 4.8 S.D. $\pm$ 1.1 S.E.M.) per cent per cm H_2O pulse pressure. The mean value for the ratio of circumferential extensibility to longitudinal extensibility was 10. (3) The abdominal aorta shortened with cardiac systole. This shortening is thought to be produced by elongating strain of the thoracic aorta. (4) The absolute magnitude of aortic wall velocities, both radial and longitudinal, was very small as compared to the blood velocity at the same site. An article has been submitted to the Journal of Applied Physiology.

Significance to bio-medical research and the program of the Institute: The findings of this study define realistic boundary conditions to

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Calendar Year 1960

Length Changes in the Aorta.

be used in the mathematical analysis of vascular dynamics.

Part B included:	Yes	No
		x

Serial No. NHI-6
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

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Individual Project Report
Calendar Year 1960

Part A

Project Title: Computation of the Aortic Ejection Velocity from the Central Aortic Pulse Pressure.

Principal Investigator: Joseph C. Greenfield, Jr , M.D.

Other Investigators: Donald L. Fry, M.D. and Samuel M. Fox III, M.D.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1
Professional: 0.5
Other: 0.5

Project Description:

Objectives: To compare the method of Fry and Mallos for measurement of the instantaneous aortic ejection velocity with the aortic ejection velocity computed from the central pulse wave.

Methods Employed: The instantaneous aortic ejection velocity will be measured by the method of Fry and Mallos. The central pulse contour will be recorded and from this the instantaneous aortic velocity will be computed using an operational amplifier. The open chested anesthetized dog will be used and cardiac output will be varied utilizing various drugs. The two methods will then be compared as to the similarity of the velocity contours.

Significance to bio-medical research and the program of the Institute: If the relationship of the central aortic pulse pressure to the aortic ejection velocity can be established, the measurement of central vascular dynamics in human subjects will be greatly simplified.

Proposed course of project: This project was delayed due to the need for better instruments, i.e. tape recorder. This equipment is now available and the project is to be continued.

Part B included	Yes	No
		x

Serial No. NHI-7
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Pathogenesis of Chronic Diffuse Obstructive Pulmonary Emphysema.

Principal Investigator: Donald P. Schilder, M.D.

Other Investigators: Donald L. Fry, M.D.

Cooperating Units: None

Man Years (calendar year 1960):
Total: .25
Professional: .25
Other:

Project Description:

Objectives: Theoretical considerations have been developed that indicate that an abnormal stress distribution within the structure of the lung may be the major factor in the production of the disruptive lesions of emphysema. The abnormal stress distribution could be the result of either congenital or acquired abnormality of the smaller terminal air passages.

Patient Material (for clinical projects); 10 normal controls from the National Institutes of Health Normal Control Group.

Major Findings: With an improved intraesophageal pressure measuring system the intrathoracic pressure may be measured at three selected sites in the esophagus simultaneously. The relationship between the oral pressure and these intraesophageal pressures was studied under static conditions in normal subjects. It has been possible to describe this relationship as a function of balloon volume, balloon position and lung volume. It seems to be independent of the rate of change of pressure or the amplitude of pressure changes. This initial part of the project, of interest in itself, is preliminary to studying the pressure differences between the three balloons under conditions of air flow, varying rates of respiration and respiratory maneuvers such as cough.

Significance to bio-medical research and the program of the Institute: If it can be demonstrated that there are significant differences in stress distribution in the lung of normal individuals as compared to those with chronic bronchitis, evidence is gained for support of

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Calendar Year 1960

The Pathogenesis of Chronic Diffuse Obstructive Pulmonary Emphysema.

the theory that the disruptive lesions of pulmonary emphysema may be the result of abnormal physical stress.

Proposed Course of Project: To study a number of normal and diseased subjects under static and dynamic conditions and complete the work on this aspect of the stress distribution in the normal and diseased lung.

Part B included	Yes	No
	x	

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Individual Project Report
Calendar Year 1960

The Pathogenesis of Chronic Diffuse Obstructive Pulmonary Emphysema.

the theory that the disruptive lesions of pulmonary emphysema may be the result of abnormal physical stress.

Proposed Course of Project: To study a number of normal and diseased subjects under static and dynamic conditions and complete the work on this aspect of the stress distribution in the normal and diseased lung.

Part B included	Yes	No
	x	

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Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Schilder, Donald P., Hyatt, Robert E. and Fry, Donald L.
An Improved Balloon System for Measuring Intraesophageal Pressure.
J. of Appl. Physiol. 14: 1057-1058, 1959.

Serial No. NHI-8
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Influence of Alveolar Gas Density and Viscosity on the Maximum Flow Vital Capacity.

Principal Investigator: Donald P. Schilder, M.D.

Other Investigators: Albert Roberts, M.D., Donald L. Fry, M.D.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1
Professional: 1
Other:

Project Description:

Objectives: Theoretically the maximum expiratory flow that can be achieved at any point in the vital capacity (maximum flow vital capacity) would be dependent on the density and viscosity of the expired gas. If it could be proved experimentally that the effect of either density or viscosity was of a minor order, it would simplify considerably the mathematical expression that has been used to describe the maximum flow vital capacity curve. It would also be of interest in view of previous studies on the effect of gas density and viscosity on the pulmonary resistance to airflow in the normal and emphysematous subject.

Patient Material (for clinical projects): 10 Normal Controls from the National Institutes of Health Normal Control Group.

Major Findings: Progress thus far has included the determination of alveolar gas composition during the performance of several maximum flow vital capacity curves. The composition varies a little during the course of a single maneuver or repeated maneuvers. Methods of accurately mixing very dense gases and determining with accuracy the viscosity of expired gases have been developed. Preliminary findings in 10 normal subjects would indicate that the maximum flow vital capacity is independent of alveolar gas viscosity but is dependent on density.

Significance to bio-medical research and the program of the Institute: The findings of this study will be used to determine more realistic coefficients to be used in the mathematical relationship describing the maximum flow vital capacity.

Serial No. NHI-8

PHS-NIH
Individual Project Report
Calendar Year 1960

The Influence of Alveolar Gas Density and Viscosity on the Maximum Flow Vital Capacity.

Proposed Course: To study several more subjects and complete the project.

Part B included	Yes	No
	x	

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Calendard Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Hyatt, Robert E., Schilder, Donald P. and Fry, Donald L. Relationship Between Maximum Expiratory Flow and Degree of Lung Inflation. J. of Appl. Physiol.13:331-336, 1958.

Fry, Donald L. The Physics of Air Flow in Emphysema. Amer. Rev. of Respiratory Diseases, 80:123-127, 1959.

Hyatt, Robert E. Pressure-Flow-Volume Interrelationships in Man. Amer. Rev. of Respiratory Diseases, 80:138-141, 1959.

Fry, Donald L. Theoretical Considerations of the Bronchial Pressure-Flow-Volume Relationships with Particular Reference to the Maximum Expiratory Flow Volume Curve. Physics in Med. and Biol.3:174-194, 1958.

Fry, Donald L. and Hyatt, Robert E. Pulmonary Mechanics. Amer. J. of Med. IN PRESS.

Serial No. NHI-9
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Relationship of the Ultra-Low Frequency Ballistocardiographic Tracing with the Time Course of Instantaneous Left Ventricular Ejection Velocity and Acceleration as Determined by the Spatial Pressure Gradient Technique.

Principal Investigator: James R. Warbasse, M.D.

Other Investigators: Samuel M. Fox III, M.D., Raymond B. Crawford, M.D., William Scarborough, M.D.

Cooperating Units: Federal Aviation Agency

Man Years (calendar year 1960):
Total: 0.2
Professional: 0.2
Other

Project Description:

Objectives: No quantitative evaluation of the ballistocardiographic recording has been possible in intact man relative to isolated left ventricular kinetic function. Because of the innocuous nature of ballistocardiographic procedures, it is hoped that a valid correlation can be made with the computed velocity and acceleration measurements as both techniques become better established.

Patient Material (for clinical projects): Those otherwise admitted for studies in the National Heart Institute.

Methods Employed: An ultra-low frequency ballistocardiographic table supplied by Dr. Scarborough has been suspended over the fluoroscopic table in the catheterization laboratory of the Section on Cardiodynamics. Its natural frequency when loaded with most of the apparatus to be used is under 4 cycles/second and its weight is approximately 15 lbs. We believe it is possible to bring the leads required by the velocity catheter technique out without distorting the ballistocardiographic signal and to record the two simultaneously on magnetic tape. No patient studies have yet been performed with the combined approach.

Major Findings: Still in an instrument development phase.

Serial No. NHI-9

PHS-NIH
Individual Project Report
Calendar Year 1960

Studies on the Relationship of the Ultra-Low Frequency Ballistocardiographic Tracing with the Time Course of Instantaneous Left Ventricular Ejection Velocity and Acceleration as Determined by the Spatial Pressure Gradient Technique.

Significance to bio-medical research and the program of the Institute: Many studies in ballistocardiography have been performed but no exact correlations with kinetic function had ever been available in intact man as noted above.

Proposed course of project: As outlined above, simultaneous studies will be performed and correlative examinations made.

Part B included	Yes	No
		x

Serial No. NHI-10
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Dopamine on Patients with Cardiovascular Disease

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: David Horwitz, M.D., Leon Goldberg, M.D.

Cooperating Units: Section on Experimental Therapeutics

Man Years (calendar year 1960):
Total: 0.1
Professional: 0.1
Other:

Project Description:

Objectives: Paralleling the initial work (reported in Atlantic City, in May 1960) covering normal controls, studies have been undertaken in patients with reasonably well-defined disease states. Three studies were undertaken during left heart catheterization with measurement of left atrial pressure both before and after left heart dye injections for cardiac output with two cases having a simultaneous radioisotope load. Two determinations were made before and two after administration of Dopamine in doses sufficient to cause a significant change in systolic arterial pressure and pulse wave characteristics.

Patient Material (for clinical projects): Patients under investigation in the general program of the Sections on Cardiodynamics and Experimental Therapeutics.

Major Findings: Cardiac output and stroke volume was increased and left atrial end diastolic pressure was the same or slightly reduced in all subjects. Attempts at the evaluation of mixing function in the left sided chambers have not been rewarding. Further studies in a variety of conditions are needed.

Significance to bio-medical research and the program of the Institute: It appears likely that there are inotropic agents of use in chronic circulatory insufficiency in addition to or supplementing the well-established use of digitalis preparations. While Dopamine and/or its precursors may represent a useful agent, no work has yet been done to establish its place in therapy.

Serial No. NHI-10

PHS-NIH
Individual Project Report
Calendar Year 1960

The Effect of Dopamine on Patients with Cardiovascular Disease

Proposed course of project: Further studies of similar nature with the possibility of combined ejection velocity determinations.

Part B included	Yes	No
		x

Serial No. NHI-11
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies Concerning the Determination and Significance of the Instantaneous Pulsatile Blood Velocity.

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: Donald L. Fry, M.D., Guy Octo Barnett, M.D., Joseph C. Greenfield, M.D., Alexander Mallos, B.S., Dali J. Patel, M.D., Ph.D.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2.5
Professional: 2.5
Other:

Project Description:

Objectives: To assess the clinical and physiologic significance and the effect of therapeutically useful agents on the ejection velocity contour of the left and right ventricles, and the flow patterns of the aorta, pulmonary arteries and distal vessels.

Patient Material (for clinical projects): Those admitted for study of circulatory inadequacy of types that would lend themselves to this procedure and specifically those with myocardial impairment rather than that associated with valvular disease.

Methods Employed: A switch has been made from passive electric analog circuits to the use of operational amplifiers (Donner Computer Units) and the incorporation of tape recording techniques for recording prime data. The use of tape recording units has allowed the recomputation of the instantaneous velocity at both real and unreal times so as to more accurately assess the contributing factors for the terms involved in the hydrodynamic equations.

Major Findings: A total of 27 patient studies have now been done with meaningful data and useful correlations being available from the majority. Some of the data has been processed through the National Institutes of Health Computer Section for Fourier analysis of harmonic content and further work in this line will be pursued. Possibly useful therapeutic agents have been tried in over a dozen of these clinical studies with interesting results which have yet to

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Studies Concerning the Determination and Significance of the Instantaneous Pulsatile Blood Velocity.

be fully interpreted.

Significance to bio-medical research and the program of the Institute: As in automotive engineering the appreciation of impaired kinetic function of the heart may best be obtained by measurement of the time derivatives of blood shift, namely, velocity and acceleration. Present means of assessing myocardial function leave much to be desired in the accurate definition of significant impairments such as may not present obvious symptoms and signs of circulatory insufficiency. A report summarizing the first 27 patient studies was presented at the American Heart Association meeting in St. Louis, October, 1960 and was apparently received with interest.

Proposed course of project: Much further work is necessary in the evaluation of the terms of the basic equations used and the range of limits within which reasonable accuracy can be obtained. Some basic work in dogs is being undertaken by Dr. James R. Warbasse and Dr. Raymond B. Crawford with the use of a Kolin sine wave electromagnetic flowmeter in a restrained aortic segment of fixed cross-sectional area. The relationship of the forces of acceleration with the spatial pressure gradient will be better defined as a result of this work and the results may lend themselves to a simpler functional index of clinical usefulness. Further work on correction circuits for catheter response and the development of catheter tip sensors are being pursued.

Part B included	Yes	No
		x

Serial No. NHI-12
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
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Part A

Project Title: The Myocardopathies: Definition of the Physiologic Disturbance and the Response to Therapeutic Agents.

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 0.2
Professional: 0.2
Other:

Project Description:

Objectives: As stated in the title, correlations will be made with objective circulatory function studies, the clinical x-ray and ECG findings and in some cases the result of myocardial biopsy which would be performed by members of the surgical clinic, NHI.

Patient Material (for clinical projects): A specific project in the assessment of the circulatory insufficiency associated with various forms of myocardopathy is a major part of the clinical activities of the Section on Cardiodynamics. Patients are admitted specifically for studies of kinetic function and other correlated investigations.

Major Findings: Twelve patients have had fairly extensive evaluations including the assessment of left ventricular ejection velocity and acceleration parameters both with and without drugs of possible therapeutic benefit. A definite correlation appears to exist between the ejection kinetic function and the performance of these same patients when exposed to exercise stress. One of these patients has had two examinations bringing out a five (5) times increase in left ventricular acceleration function during a period when clinical improvement was associated with only a small change in stroke volume and calculated arterial venous oxygen extraction determinations during rest and exercise. Some of these cases have had left heart studies to define the left ventricular pressure status.

Significance to bio-medical research and the program of the Institute: As objective measurement of heart function becomes more frequent there is a growing realization of the number of cases of myocardial

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The Myocardopathies: Definition of the Physiologic Disturbance and the Response to Therapeutic Agents.

impairment in clinical practice. There are definite needs to more accurately determine the significance of this impairment in clinical practice, industrial, insurance, rehabilitation and military medical service.

Proposed course of project: Continuing with more refined techniques as outlined.

Part B included	Yes	No
		x

Serial No. NHI-13
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Characteristics and Possible Usefulness of Left-Sided Isotope Injections with Precordial Time Activity Scanning.

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 0.2
Professional: 0.2
Other

Project Description:
Objectives: To determine what if any useful information is specifically available with the precordial scanning technique after left-heart injections (left atrium and left ventricle) in various disease states.
Patient Material (for clinical projects): Those with the routine indications for left heart studies.
Methods Employed: An uncollimated but laterally shielded 2" scintillation crystal was placed just clear of the anterior chest (precordium) and 9 to 15 microcuries injected with a special device through a PE 50 catheter into the left ventricle or through the standard Ross Trans-septal needle into the left atrium. Using the shortest available time constant (0.05 seconds) curves were inscribed on a Texas rectiriter, the time constant of which was unfortunately considerably longer.
Major Findings: From eight (8) patient studies, curves were examined for the alleged alteration in downslope reported by Sevelius and Johnson to represent the "coronary circulation". No such break was found. An examination was also made for constancy of the count decrement divided by the total value ($\Delta C/C$) which might be considered to give an indication of the relation of each stroke ejection from the total circulatory pool being viewed. It was found that there was up to a 40% variation in this ratio during such parts (2 to 3 ejection cycles) of the downslope of the activity curve as might be considered as a period of "exponential wash-out". The simultaneously

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The Characteristics and Possible Usefulness of Left-Sided Isotope Injections with Precordial Time Activity Scanning.

injected indicator dye was used for the determination of cardiac output during the same approximate interval. The hope that the combined technique would lead to a reasonably useful assessment of end-systolic and end-diastolic volumes in the left ventricle does not yet appear justified.

This study was undertaken independently of a similar study later presented by Drs. Andre Cournand and Luigi Donato at some meetings last spring. Although Dr. Donato's careful approach to this problem is reassuring relative to the eventual usefulness of the approach, all his determinations were done on the right side of the heart.

Significance to bio-medical research and the program of the Institute: Little is known of the effect of therapeutic approaches on the heart volume and the related tension required to produce effective circulatory action in many normal and abnormal conditions in man. A technique giving a useful index or preferably an exact measurement of the functional volumes involved would be of obvious value.

Proposed course of project: After a full morning's frank exchange of views with Dr. Donato in his laboratory, it appears that the approaches pursued independently and without knowledge of each other's efforts are incomplete in both laboratories. It is probable that neither group will get highly significant data until a more accurate measurement is available of both the contribution of streaming upon ventricular mixing and the geometric factors involved with surface counting.

Part B included	Yes	No
		x

Serial No. NHI-14

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Derivation and/or Improvement of Methods for Study of the Human Circulatory Response to Exercise and Other Maneuvers

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: Hubert Hofmann, M.D., Raymond B. Crawford, M.D., James Richard Warbasse, M.D., Arthur S. Lynn, B.S.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 0.2
Professional: 0.2
Other:

Project Description:

Objectives: To find methods requiring little time and/or patient inconvenience that will give a useful assessment of circulatory function.

Patient Material (for clinical projects): Patients under examination and treatment in other projects in the Clinical Center.

Major Findings: A grossly simplified yet accurate method of calibration for dye dilution studies has been worked out. Multiple studies on patients have demonstrated its reliability. A relatively simple injection unit for rapid, repeated and accurate injection of indicator substances has been designed, tested and found useful. Definition of the exercise response characteristics has been assessed in 24 human subjects with various forms of myocardial insufficiency permitting differentiation of others relative to preliminary limits of apparent normality. Some tentative definition has been made of the limitations of "Beer's" Law relating the effect of dye concentration on the light transmitting characteristics of whole human blood. In contrast to some recent reports stroke volume has been found to frequently rise upon supine exercise.

Significance to bio-medical research and the program of the Institute: The definition of the normal and borderline abnormal response to exercise may well represent as simple and useful a test as any for the evaluation of the large population of persons with possible myocardial

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The Derivation and/or Improvement of Methods for Study of the Human Circulatory Response to Exercise and Other Maneuvers

insufficiency. Present methods do not appear adequate.
Proposed course of project: Further work is needed relative to the Beer's Law definition as well as to better establish normal response patterns.

Part B included	Yes	No
		x

Serial No. NHI-15
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Definition of the Localization and Extent of Myocardial Cell Damage by External Scanning after Injection of Radioactive Substances.

Principal Investigator: Raymond B. Crawford, M.D.

Other Investigators: Samuel M. Fox III, M.D., John R. Howley, B.S., William C. Roberts, M.D.

Cooperating Units; Department of Pathology, NIH; Plant Safety Branch, OD

Man Years (calendar year 1960):
Total: 0.1
Professional: 0.1
Other

Project Description:
Objectives: It appeared likely to Dr. Crawford that some substances suitable for radioactive isotope labelling might be selectively retained in areas of necrosis and/or cell death in the myocardium to such an extent that external scanning techniques after the injection of isotopes would localize and perhaps give some quantitative indication of the extent of the altered tissue. If such were the case the use of short acting isotopes in relatively innocuous doses might give the clinician useful information on the progress of cell damage and the rate of healing.
Patient Material (for clinical projects): Studies so far have not involved patient material, although this would be contemplated if the basic studies continue to be encouraging.
Methods Employed: Small amounts of radioactive cobalt and silver have been injected into dogs in which the anterior descending coronary artery has been ligated previously. At the time of sacrifice of the dogs, pathologic sections have been taken after surface scanning has been done with reasonably good collimation. As the work progresses, external scanning is contemplated and more accurate assessment of the electrocardiographic changes might be included.
Major Findings: It is found that cobalt has disintegrations of such power that discrimination cannot be made with the standard collimators. Silver, on the other hand, has not yet been found to accumulate in the areas of necrosis to the degree that would be desirable for discrimination.

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Radio-iodine has been used by others for similar work with concentrations of 3:1 to 4:1 between infarcted and normal myocardium. It appears that iodine, for reasons which are not particularly obvious, may represent the best of the commonly available isotope materials.

Significance to bio-medical research and the program of the Institute: The magnitude of the coronary artery disease problem appears to be sufficient stimulus for almost any reasonably safe and possibly promising investigation which would more accurately define the state of a patient where coronary insufficiency and/or myocardial cell death is suspected.

Proposed course of project: Continuation in the present direction as long as developments appear to warrant the time and effort required.

Part B included	Yes	No
		x

Serial No. NHI-16
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
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Part A

Project Title: Aldosterone secretion rate in primary and secondary aldosteronism; the role of aldosterone in edema

Principal Investigators: Bartter, F.C., M.D., Bell, N.H., M.D., and Kliman, B., M.D.

Other Investigators: Delea, C. and staff

Cooperating Units: Gilbert, J., M.D., Surgery Branch, NHI

Man Years (calendar year 1960)
Total: .979
Professional: .534
Other: .445

Objectives: To determine the secretion of aldosterone in patients with aldosteronism as compared with that in patients and normal subjects with secondary aldosteronism. The effects of expansion of intravascular volume with albumin, of sodium deprivation and sodium loading, and of aldosterone antagonists are being measured in both forms of aldosteronism. The early and late effects of ACTH on the secretion of aldosterone are being studied and compared with the effect on urinary aldosterone.

Methods Employed:

The balance technic has been employed throughout. Patients with primary aldosteronism were studied before and after surgery for removal of the immediate cause of the over-secretion of aldosterone.

The secretory rate of aldosterone in man is subject to direct measurement Tritium labeled aldosterone is given intravenously. A timed urine collection is obtained, usually over 24 hours. A urinary metabolite of aldosterone is isolated, and a second radioactive label is applied by acetylation with C^{14} labeled acetic anhydride. Purification provides a final sample which contains metabolite formed from the tracer dose diluted with endogenous aldosterone. The change in specific activity of the tracer aldosterone is a measure of its dilution and the endogenous secretion rate can be calculated.

Major Findings:

Studies in 8 patients with primary aldosteronism have shown that: (1) Sodium deprivation may produce potassium retention and provide a means whereby primary aldosteronism may be distinguished from aldosteronism secondary to sodium losing renal disease. (2) Aldosterone antagonists may induce significant increases of aldosterone secretion in primary aldosteronism. When this effect occurs, there is always significant retention of potassium and increase in serum potassium. Because of this effect on potassium the antagonists do not provide a means of distinguishing between primary and secondary aldosteronism. (3) Infusion of albumin, which regularly decreases aldosterone secretion in normal subjects and in subjects with secondary aldosteronism, does not decrease aldosterone secretion in patients with primary aldosteronism. Two patients with tumors were found to secrete over 700 μg a day before infusion of albumin and to secrete the same amount after a course of albumin which effectively expanded intravascular volume.

Normal volunteers were found to secrete from 100 to 300 μg of aldosterone a day and this increased two- to four-fold with the development of sodium loss due to restriction of intake. These figures are to be compared with the approximately 10-fold increase of urinary aldosterone which occurs in normal subjects upon restriction of dietary sodium. The effect of similar procedure on secretion of aldosterone in patients with primary aldosteronism is under investigation.

Proposed Course of Project:

Patients with cirrhosis, idiopathic edema, cardiac failure, renal tubular acidosis, adrenogenital syndrome, and other disorders are under study by a similar protocol; normal, sodium depleted subjects are being studied for comparison.

The relation of arterial pulse pressure to aldosterone secretion, which has been documented in dogs, is now being studied in these human subjects before and after treatment. Treatment with Aldactone (aldosterone antagonist) results in sodium loss and a secondary increase in aldosterone secretion. This phenomenon may also occur with diuretic agents. Since only some cases of pathologic retention of fluid and sodium respond to aldactone therapy, the role of secondary hyperaldosteronism in these conditions is under investigation with this agent and other pharmacologic agents.

Two manuscripts are in progress, one dealing with diagnostic studies and one with surgical management of primary aldosteronism.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Bartter, F.C., M.D. The Clinical Use of Aldosterone Antagonists. Chas. C. Thomas, pub. 211 pages. 1960 . Dr. Bartter edited book and wrote chapter entitled "The mode of Action of Spirolactones and their use in the Diagnosis of Aldosteronism, pp. 2-13.

Goldsmith, R.S., Meroney, W.H., and Bartter, F C. Prominent Peripheral Edema Associated with Primary Aldosteronism Due to an Adrenocortical Adenoma. J. Clin. Endocrin. and Metab. 20: 1168-1183, 1960

Bartter, F. C. Secondary Aldosteronism. Clin. Endocrin. 1: 392-396, 1960.

Honors:

Delivery of annual Musser lecture at Tulane University School of Medicine by Dr. F. C. Bartter entitled "Aldosterone and Edema".

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1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in idiopathic osteoporosis

Principal Investigators: Bartter, F.C., M.D., Bell, N.H., M.D., and McSwiney, R.R., M.B.

Other Investigators: Delea, C. and staff

Man Years (calendar year 1960)
Total: 2.080
Professional: 1.451
Other: .629

Objectives:

Idiopathic osteoporosis is defined for the purposes of these studies as osteoporosis in which neither androgen nor estrogen induces a positive calcium balance. These studies were undertaken to determine the defect in osteoid synthesis in patients with this disorder and to investigate in particular the mechanism by which human albumin given intravenously induces positive calcium balance which is far greater in extent and of much greater duration than that which can be explained as simple binding to the albumin. Other forms of therapy are being investigated.

Methods Employed:

Patients with idiopathic osteoporosis have been studied on balance regimen. At the outset it is determined that the patient does not respond to androgen and estrogen as a prerequisite for the diagnosis of idiopathic osteoporosis. The patients are then subjected to a number of stimuli while measurement of calcium balance is continued. Patients are given at intervals oral doses of Ca^{47} and the gamma ray count is done on subsequent stools until it reaches zero. In this way a measure of calcium absorption is achieved independent of that resulting from the balance study itself. Ca^{47} is also used intravenously to determine the rate of bone formation, after the manner of Bauer: the effect of therapy can thus be measured "directly."

Major Findings:

To investigate the hypothesis that albumin may have its effect because of its oncotic properties, dextran was given to patients with

idiopathic osteoporosis. The results were equivocal, in that one patient developed a fall of urinary calcium in response to both albumin and dextran, a second patient showed no response to either regimen. (This patient was indeed the only one we have studied who did not show a response to albumin.) In two other patients albumin produced a clear-cut positive calcium balance.

Three patients were given parathyroid extract at intervals with the hope that the rebound calcium retention would be more extensive than the calcium loss with treatment. No appreciable positive calcium balance was obtained with this regimen.

Two patients with idiopathic osteoporosis were given strontium in large doses in an effort to determine whether the strontium could itself be deposited into apatite and whether it induced positive calcium balance as previously reported. These studies are still under analysis, but preliminary results suggest no effect from strontium.

Proposed Course of Project:

Further studies on the effects of dextran, albumin and strontium are in progress.

Part B included No

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1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3 Bethesda, Md.

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Individual Project Report
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Part A

Project Title: Studies on the mechanism of development of experimental atherosclerosis

Principal Investigator: Duncan, L.E., Jr., M.D.

Other Investigators: Buck, K. and Lynch, A.

Man Years (calendar year 1960)
Total: 3.00
Professional: 1.00
Other: 2.00

Objectives:

The object of this project is to develop detailed information on the process involved at the arterial level in the development of atherosclerosis.

Methods Employed:

Early in the course of the work it was necessary to develop an experimental procedure and mathematical analysis that would make possible the determination of the rates of entrance of proteins into aortic tissue. This problem was solved and the procedure has been used extensively in the subsequent work.

Patient material: None

Major Findings:

A brief statement will be made of findings of this project prior to this year in order to clarify the significance of the work of the past year.

The rates of movement of plasma albumin into and out of the aortic wall of the dog were determined. Albumin was found to enter the aortic tissue fastest in the upper aorta and progressively less rapidly down the length of the aorta.

It seemed likely that albumin and plasma lipoprotein might enter the aortic wall by the same mechanism and thus show the same gradient

of rates. The combination of this possibility and the filtration theory of atherosclerosis led to the prediction that there might be a longitudinal gradient in the deposition of cholesterol in the aorta during the development of atherosclerosis. The prediction was tested experimentally and confirmed; there is a striking gradient in the rate of deposition of cholesterol in the aortic wall in experimental atherosclerosis, and the gradient is very similar to that of the entrance rates of albumin. These data support the filtration theory and the supposition that in the development of atherosclerosis lipoprotein enters arterial walls by a mechanism similar to that by which albumin enters.

It thus seemed important to learn something about the mechanism by which albumin enters aortic wall and the factors that influence it. It also seemed important to get more direct information on the movement of lipoprotein movement into arterial walls. Our work of the past year has been concerned with these areas.

We first investigated the possibility that the gradient was due to some dynamic aspect of blood pressure or blood flow, or to respiratory variations in intrathoracic pressure. We did this by studying the passage of albumin into aortas removed from the body and filled with plasma under a normal mean arterial pressure. This preparation is not entirely satisfactory since the static conditions lead to maintenance of arterial blood pressure in the capillaries of the aortic wall. However, there is sufficient preservation of the gradient under these circumstances to exclude phasic pressure variations as its cause.

Since in the individual aorta the rate of entrance is directly proportional to aortic width, it seemed possible that the width is a determinant of the entrance rate. This was rendered plausible by the fact that circumferential tension is directly proportional to aortic width. This possibility led to an investigation of the rates of entrance of albumin into the aortic walls of large and small dogs. The data showed that the rate of entrance at any given level along the aorta is not proportional to the aortic width. Thus the generalization that the rate of entrance is directly proportional to the aortic width is not true.

The effect of blood pressure on the entrance of albumin into the aortic wall has been studied. The data are not yet complete but those we have are sufficient to show that pressure has a great effect on the entrance rate. In the ascending aorta a tenfold increase in the entrance rate has been produced by elevating the blood pressure.

Two methods of obtaining more direct information on the entrance of lipoproteins have been tried. Canine lipoproteins have been labeled with radioactive sulfur by feeding labeled yeast to dogs with high plasma lipoprotein levels. However, we have found the lifetime of these labeled lipoproteins to be so extremely short in the hyperlipemic dog that it appears very unlikely that their passage into aortic wall can

be studied. Labeling of the lipid components of lipoproteins would not lead to interpretable results since there is considerable exchange of the lipid components between high and low density lipoproteins. We have therefore set aside labeling techniques and have been trying to detect lipoproteins in the aortic wall during the development of experimental atherosclerosis using the analytical ultracentrifuge for the demonstration of these lipoproteins. With this technique we have succeeded in demonstrating the normally occurring lipoproteins of aortic tissue, but have been unable to demonstrate the presence of additional lipoproteins early in the course of experimental atherosclerosis. We are now trying to demonstrate their presence in the later stages of atherosclerosis.

Significance to medical research:

These studies are leading to an increased understanding of the processes involved in the development of atherosclerosis.

Proposed Course of Project:

There are a great many aspects of this project that might be profitably pursued. The two most important things to do now seem to be to study the entrance of lipoproteins more directly and to study the mechanism of the gradient.

It is becoming more likely that the gradient is due to a progressive change in the structure of the aortic wall down its length. The intimal endothelium rests on ground substance and a framework of connective tissue. This framework undergoes striking changes down the length of the aorta which may explain the gradient. It seems probable that studies of this structure may lead into the area of age changes in arterial walls that render them prone to the development of atherosclerosis.

Part B Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Duncan, L.E., Jr., Cornfield, J., and Buck, K.: Circulation of iodinated albumin through aortic and other connective tissues of the rabbit. Circulation Research 6: 244-255, 1958.

Duncan, L.E., Jr., Cornfield, J., and Buck, K.: Circulation of labeled albumin through the aortic wall of the dog. Circulation Research 7: 390-397, 1959.

Duncan, L.E., Jr. and Buck, K.: Passage of labeled cholesterol into the aortic wall of the normal dog. Circulation Research 7: 765-770, 1959.

Duncan, L.E., Jr. and Buck, K.: Quantitative analysis of the development of experimental atherosclerosis in the dog. Circulation Research 8: 1023-1027, 1960.

Duncan, L.E., Jr. and Buck, K.: Comparison of the passage of labeled albumin into canine aortic wall *in vivo* and *in vitro*. Am. J. Physiol. In press.

Serial No. NHI-19
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Study of Calcium Homeostasis in the Parathyroidectomized Dog

Principal Investigator: Alexander, R. L., Jr., Ph.D.

Other Investigators: Casper, A.G.T.

Man Years (calendar year 1960)
Total: 1.20
Professional: 1.00
Other: .20

Objectives:

Although evidence accumulated thus far has established that parathyroid hormone regulates calcium homeostasis in the body essentially by its action on bone and kidney tissue, the mechanism of this action is still unknown. Studies in recent years involving the use of radioisotopes of calcium and phosphorous in normal and parathyroidectomized animals suggest that at least two different fractions of bone mineral exist--a so-called labile fraction arising from newly formed surface layers of bone and a more stable fraction located in the deeper, more compact areas of bone. A survey of existing data has led some investigators to propose that the labile fraction of bone maintains the plasma calcium at a level of approximately 5-7 mg. per 100 ml. by means of a passive physico-chemical equilibrium of this fraction of bone with circulating body fluids. It is further proposed that the action of parathyroid hormone on the stable fraction of bone mineral causes the continuous release of sufficient calcium to elevate the plasma calcium to a level of about 10 mg. per 100 ml., the steady state existing in the normal blood picture. A reduction of the calcium ion concentration in the plasma is believed to provide a stimulus to the parathyroid glands, which immediately secrete more hormone, bringing about an increased release of calcium from the stable fraction and a consequent increase in the calcium level of the plasma. Further, it is proposed that the parathyroid mechanism, while responsible for hour-to-hour or day-to-day adjustments, is not alone adequate for the minute-to-minute interplay between blood and bone. Minute-to-minute adjustments in plasma calcium level are believed to be brought about by diffusion between blood and bone, occurring independently of parathyroid action.

While it is clear that the parathyroid glands are necessary for the maintenance of normal plasma calcium levels, the mechanism which effects the transfer of calcium from bone to fluids in the absence of the parathyroid glands has not been well understood. Most of the experiments bearing on this problem have been concerned with the mobilization of calcium from bone after the blood has been partially depleted of this element. There has been general disagreement among investigators concerning the rate at which bone is able to release calcium to the body fluids of the parathyroidectomized animal. Some have claimed that the rate of release is the same as that of the normal animal, while others have presented evidence to indicate a much slower rate as a result of deficiency in parathyroid hormone. It is the purpose of the present work to attempt to resolve this conflict.

It has been demonstrated earlier that ethylenediaminetetraacetic acid (EDTA) can form a soluble chelate with ionic calcium at physiologic pH, thereby serving as an effective agent for decreasing the concentration of calcium present in circulating body fluids. The extent to which the concentration is reduced depends upon the dosage of EDTA, the rapidity with which it is administered, and the route of administration.

In the present study the plasma calcium concentration in normal and thyroparathyroidectomized dogs was lowered by intravenous infusion of a constant dose of EDTA over a period of two hours. Following the infusion, the calcium concentration was measured at 20-minute intervals in order to determine the rate at which preinjection levels of plasma calcium would be attained. Previous studies of this type in nephrectomized animals maintained on a low-calcium diet indicate that changes in plasma calcium levels following induced hypocalcemia can occur independently of renal or intestinal influence. Changes in plasma calcium levels following partial depletion of blood calcium can therefore serve as a measure of the ability of bone to supply calcium to the body fluids.

Major Findings:

Various studies in the past have shown that the existing pool of bone salts in young animals is more readily available than that of the adult, essentially as a result of the higher percentage of cancellous bone present and the more vascular nature of immature bone. This was verified in the present work by a comparison of the changes in plasma calcium levels in normal and immature dogs following the intravenous infusion of increasing dosages of EDTA.

If parathyroid hormone exerts no effect on the rate at which calcium is mobilized from bone, as has been reported earlier, then one must assume that the response of the parathyroidectomized animal to a specific dose of EDTA will be identical to that obtained in the intact

animal. Eight puppy dogs maintained on a low-calcium diet (0.02 per cent) were infused with a concentration of EDTA sufficient to chelate 4 mg. of calcium per Kg. per hour. A comparison of changing plasma calcium levels during the infusion revealed that the parathyroidectomized animal could not mobilize calcium at the same rate as that obtained prior to removal of the parathyroid glands. Furthermore, the preinjection calcium levels were reached in the normal animals within a period of one hour or less following the end of the infusion, whereas in the parathyroidectomized animal preinjection levels were reached in some animals only after periods up to 24 hours, and in others not at all.

Experiments currently in progress in adult dogs maintained on the low calcium diet have shown in four animals that the same decreased ability of the parathyroidectomized animal to mobilize calcium exists as that found in puppy dogs. It has also been demonstrated in small numbers of both immature and mature parathyroidectomized dogs that the response to EDTA infusion is restored almost to normal 18 hours following the subcutaneous injection of 50 units/Kg. of parathyroid extract.

In an attempt to reveal something of the effect of vitamin D on the body stores of calcium, three parathyroidectomized adult dogs were placed on a daily dose of 20,000 units/Kg. of vitamin D, and, as soon as the plasma calcium levels had returned to normal, the response to EDTA-induced hypocalcemia was measured. The results of this work indicate that the vitamin can adequately take over the calcium-mobilizing function of the parathyroid hormone. The same response was obtained with vitamin D in two immature parathyroidectomized dogs using a daily dose of 3,000 units/Kg. to restore normal calcium levels.

In conclusion, the evidence obtained in these studies suggests that the recovery phase of plasma calcium concentrations following an EDTA-induced hypocalcemia is a function of the action of parathyroid hormone on the mobilization of bone mineral. Whether the reduced ability of the parathyroidectomized dog to mobilize bone calcium is brought about by a reduction in the available pool of calcium and/or by loss of an effect of the hormone on the labile fraction of bone cannot be ascertained from the evidence presented. It is of interest that a recently reported study on human patients suffering from hypoparathyroidism but having normal plasma calcium levels revealed that the recovery of calcium levels to normal following an EDTA-induced hypocalcemia required a period of about 18-24 hours as compared with an 8-12 hour period in normal patients receiving the same dose of EDTA. These findings give added support to the results of the present work.

Proposed Course of Project:

It is proposed to extend and amplify the present studies with

parathyroid extract and vitamin D. In addition, studies on the clearance of calcium will be instituted.

Part B included No

Serial No. NHI-20
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

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Part A

Project Title: The effect of the parathyroids on renal excretion of phosphate

Principle Investigators: Bartter, F.C., M.D. and Delea, C.

Other Investigators: Casper, A. and Palumbo, R.D.

Man Years (calendar year 1960)
Total: .984
Professional: .173
Other: .811

Objectives:

All established phenomena concerning renal phosphate excretion in man and in the dog can be explained on the assumption that phosphate is filtered at the glomerulus and partially reabsorbed in the tubules. There are many suggestions in the literature, however, to the effect that phosphate is secreted by the renal tubules Until it is established whether secretion of phosphate occurs in man or dog, a final interpretation of the mode of action of parathyroid extract on phosphate excretion in these species is not possible.

Methods Employed:

Renal clearances were performed on dogs during which phosphate filtered and excreted were measured and phosphate reabsorbed (or secreted) was calculated. The effects of ACTH and Cortisone, of severe acidosis, and of parathyroid extract, were examined. The effect of parathyroid extract on Tmp was also studied in parathyroidectomized dogs and in patients with hypoparathyroidism.

Major Findings:

1. Tubular secretion of phosphate has not been established for the dog.

2. A Tm for phosphate is readily established in dog and man, phosphate reabsorption remaining constant over a wide range of filtered phosphate load. This finding provides indirect evidence against significant tubular secretion of phosphate.

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3. The effect of parathyroid extract in increasing phosphate excretion can be shown to occur exclusively at the expense of reabsorbed phosphate.

Proposed Course of Project:

To study the effect of glucose 6-phosphate on phosphate secretion; to study the excretion of phosphate in distal tubules utilizing the stop-flow technic.

Part B included Yes

Serial No. NHI-20
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Bartter, F.C. The Effect of the Parathyroid on Phosphate Excretion. "Symposium on Parathyroid Research Trends, Rice Institute, 1960". Ed. Dr. R. O. Greep

Serial No. NHI-21
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in idiopathic edema

Principal Investigators: Bartter, F.C., M.D.

Other Investigators: Delea, C. and staff

Man Years (calendar year 1960)
Total: .875
Professional: .173
Other: .602

Objectives:

A large number of reports contain vague descriptions of female patients with greater or less tendency to soft tissue swelling and weight gain on a roughly cyclic basis which can be shown not to be the result of known cardiac, renal or hepatic disease. In some it is suggested that the cycles are related to the menstrual cycle; in others the syndrome continues without relation to menstrual cycle or after hysterectomy. In some it has been found that aldosterone secretion was inappropriately high for the sodium intake or for the total body sodium.

The syndrome has been studied by placing patients thought to have the disease on balance regimen and measuring the response to desoxycorticosterone, to sodium deprivation, and to sodium loading, with especial reference to aldosterone secretion during the latter two procedures. In addition, the effect of extensive upright posture as opposed to extensive bed rest has been observed. Finally, an attempt has been made to document the normal circadian rhythms associated with sodium retention or loss in an effort to throw light on the possible abnormal rhythms found in patients with a disorder.

Major Findings:

Almost without exception it has been found that the patients with the disorder lose the tendency to sodium retention when admitted to the hospital and placed on constant regimen. Patients with documented edema formation in the past history have shown the ability to ingest 400 mEq of sodium a day without increase of body weight or positive

sodium balance. The preliminary results suggest that patients with a disorder retain more sodium upon administration of desoxycorticosterone than do normals on a similar regimen. In view of the limited information as to the normal behavior in this regard, the findings must be considered preliminary.

Seven normal girls were studied for circadian rhythms. The variation of body temperature as determined with calibrated thermometers was measured and compared with the rhythm for secretion of hydrocortisone, aldosterone, and for rhythmic variations in blood eosinophils and polymorphonuclear leucocytes, urinary sodium, chloride, potassium, osmolality, and aldosterone. The studies are still being documented. This project is being carried out in conjunction with Dr. Franz Halberg of the University of Minnesota whose technicians assisted with the blood determinations and who is at present subjecting the results to computer analysis.

Proposed Course of Project:

A similar group of females who have been for a number of years inmates of an institution for the feeble-minded will be studied according to a similar protocol. The pattern in these subjects should contain much less "noise" than that of the students already studied in that their living patterns are subject to extreme regularity and they have few emotional disturbances. It is further planned to extend exactly similar observations to patients with the history or findings of idiopathic cyclic edema.

Part B included No

Serial No. NHI-22
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on aldosterone secretion in fasting

Principal Investigators: Bartter, F.C., M.D. and Bell, N.H., M.D.

Cooperating Investigators: Bloom, W., M.D., Dir. of Med. Edu., Piedmont Hospital, Atlanta, Ga.

Man Years (calendar year 1960)

Total:	1.104
Professional:	.284
Other:	.820

Objectives:

The literature contains numerous references to the salt loss of the fasting state and this has been noted in several studies from this laboratory. It has been proposed that the salt loss of the fasting state exceeds significantly that of sodium deprivation alone. The present studies were organized to determine 1.) whether significantly more sodium is lost consistently with fasting than is lost with sodium deprivation and 2.) whether secretion of aldosterone is lower with fasting than it is with sodium deprivation, and 3.) whether re-feeding the fasting subject with pure carbohydrate or fat, selectively affects sodium retention and aldosterone secretion. These studies have been carried out in conjunction with Dr. Walter Bloom of Atlanta, Georgia who has sent a number of the patients and who has measured with us the urinary ketone bodies in the fasting state.

Methods Employed:

Obese subjects have been studied on the metabolic ward before and during total fast and subsequently during the replacement of a single foodstuff.

Major Findings:

Four studies have been carried out to date. In all four subjects the sodium loss with total fasting was strikingly greater than the sodium loss during a comparable period of salt deprivation. In two of the subjects it appeared that the loss was indeed associated with lower

values for urinary aldosterone than are normally seen with sodium deprivation. In one of the subjects the effect of sodium deprivation was to induce a rise of aldosterone twice as great as that found with fasting. All patients showed, as expected, a strikingly negative potassium balance with fasting which was not, of course, seen with sodium deprivation. In only one did the serum potassium fall significantly.

Proposed Course of Project:

The studies will be extended to include more subjects in the hope that a pattern may arise relating sodium loss, aldosterone secretion, and either potassium loss or decrease of serum potassium in the fasting as opposed to the simply sodium-depleted state. If such a pattern arises, the new variable relating to the control of aldosterone secretion may appear in that it should become apparent which food is necessary to bring back the lowered aldosterone secretion of the fasting state.

Part B included No

Serial No. NHI-23
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Role of vitamin D in the pathogenesis of metabolic bone disease of renal failure

Principal Investigators: Bell, N.H., M.D., Bartter, F.C., M.D.

Other Investigators: Delea, C. and staff

Man Years (calendar year 1960)
Total: 1.163
Professional: .534
Other: .629

Objectives: To investigate the effect of aluminum hydroxide, of vitamin D and of alkali on calcium absorption and on calcium balance in patients with renal osteitis and in normal subjects. It has been postulated that excessive intestinal phosphate excretion in patients with renal failure may impair calcium absorption by binding calcium. Aluminum hydroxide, by binding phosphate, should make available more calcium for absorption and thus correct the defect.

Methods Employed:

Patients are studied on balance regimen. To a basic neutral ash diet sodium bicarbonate, amphogel, and vitamin D are added according to a form of Latin square design. On each regimen a known dosage of Ca^{47} is fed by mouth, and the recovery is measured in the stools, collections being continued until recovery is zero on a given day. The study is carried out as a balance study and in addition indices of bone formation such as serum calcium and phosphorus and alkaline phosphatase are determined at regular intervals.

Major Findings:

Metabolic studies to date in two patients with renal osteitis and two normal subjects suggest that amphogel may have no effect on calcium absorption. In the two patients, vitamin D increased calcium absorption, with rise of serum calcium, and the bone disease subsequently improved, suggesting that secondary hyperparathyroidism was suppressed.

Proposed Course of Project:

Further studies are in progress to support the previous findings.

Part B included No

Serial No. NHI-24
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Role of ADH in salt and water metabolism

Principal Investigators: Bartter, F.C., M.D., and Saucier, G., M.D.

Other Investigators: Hellman, E., M.D. (NCI) and Schwartz, W., M.D. (N. Eng. Center Hosp. and Tufts Univ. Sch. of Med.)

Man Years (calendar year 1960)

Total:	.816
Professional:	.673
Other:	.143

Objectives:

To investigate the effect of ad libitum and controlled water intake on sodium excretion in patients under the effect of Pitressin. It has been postulated that in patients with bronchogenic carcinoma hyponatremia can result from sustained inappropriate secretion of antidiuretic hormone (ADH). It has also been denied that ADH alone would induce the changes of the syndrome. The present studies were designed to evaluate the relationship of the water intake to the sodium loss, and the role of voluntary water intake.

Methods Employed:

1.) Blind studies were carried out in which subjects receiving ADH were unaware of the nature of medication so that their spontaneous drinking or rejection of water could be measured, and 2.) subjects receiving constant doses of pitressin tannate in oil were given graded doses of water so that a steady state could be established at different levels of serum sodium. 3.) Reported cases of inappropriate secretion of ADH have been reviewed and in many cases the reporting doctor has been consulted for further information. 4.) Two patients showing inappropriate secretion of ADH resulting from acute intermittent porphyria have been studied.

Major Findings:

The results continue to indicate 1.) that normal subjects unaware of the nature of the medication will continue to drink water while receiving Pitressin and that a decrease of thirst does not protect a

subject from the noxious consequences of inappropriate continued secretion of ADH.

2. It was found possible to establish the steady state at several different levels of serum sodium by careful control of water intake. At each steady state the patient achieved sodium balance but an increase of water intake could move the patient's serum sodium osmolality to a low level before a new steady state was established. This state in turn could be displaced by a lower one with further increase of water intake.

3. A number of cases of inappropriate secretion of ADH have now been reported and this material is now being assembled for publication in review form.

4. It appears that acute intermittent porphyria is an additional cause for inappropriate secretion of ADH and that in this syndrome the combination of sodium loss with hyponatremia and urine osmolality inappropriately high for the serum osmolality is not infrequently seen.

Proposed Course of Project:

To continue to induce hyponatremia in normal subjects and to accumulate more data on patients with this syndrome.

Part B included Yes

Serial No. NHI-24
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Schwartz, W.B., Tassel, D. and Bartter, F.C. "Further Observations on Hyponatremia and Renal Sodium Loss Probably Resulting from Inappropriate Secretion of Antidiuretic Hormone". N. Eng. J. Med. 262:743-748, 1960

Serial No. NHI-25
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Biosynthesis of $4C_{14}$ Aldosterone

Principal Investigators: Kliman, B., M.D., Kaplan, N.M., M.D., and Lewis, W.J.III

Other Investigators: None

Man Years (calendar year 1960)
Total: 1.50
Professional: 1.50
Other: 00

Objectives and Major Findings:

Incubation of beef adrenal capsule slices with $4C_{14}$ corticosterone has resulted in a 20 per cent yield of $4C_{14}$ aldosterone. Purification with paper chromatography revealed the presence of two radioactive unknowns which migrated near the aldosterone. These substances could not be found in the substrate sample and did not appear during storage of each of these three steroids. Characteristic acetate derivatives could be formed and when tritium labeled acetate was used the number of acetylable groups was found to be one and three for the unknowns, whereas aldosterone can accept two, and corticosterone one acetate group. The unknowns appear to be stable during storage and may represent intermediates in the biosynthesis or decomposition products of either aldosterone or corticosterone. The former seems likely, since it has been possible to detect these unknowns in adrenal incubation without addition of precursor corticosterone.

Large scale incubations are being done to provide material sufficient for chemical analysis of the unknowns.

The ring labeled C_{14} aldosterone has been obtained for use as a tracer and has a specific activity of 0.04 μc/μg. It has been useful as an internal standard which can be added to assay samples prior to purification. The tracer is stable and is readily detected and measured.

The method has been extended to develop a bioassay for in vitro stimulation of aldosterone secretion. Multiple small specimens have been incubated with $4C^{14}$ corticosterone or $4C^{14}$ progesterone and the steroids produced isolated by chrometography and measured by C^{14} count. In this way it was possible to test the effect of Infundin, of ACTH, and of various extracts of canine dog brain on the secretions of aldosterone in vitro. A similar procedure is being employed for measurement of in vitro effects of renin and hypertensin.

Proposed Course of Project:

The clinical use of the tracer will soon be tested in isotope dilution studies. The $4C^{14}$ aldosterone is being employed in the double derivative isotope dilution method to provide a tracer whose recovery can be measured and is thus not dependent upon completeness of extraction or completeness of acetylation. The small sample method is being extended to document further the effect of renin and hypertensin and to measure the effects of other possible stimuli to aldosterone secretion in the in vitro system. The determination of total as well as radioactive aldosterone is being added to these studies to clarify the findings further.

Part B included No

Serial No. NHI-26

1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The role of adrenal corticosteroid in the salt retention of the edematous state

Principal Investigators: Bartter, F.C., M.D., Gann, D.S., M.D., Cruz, J. M.D.

Other Investigators: Casper, A., Henderson, H.H., Smith, H.C.

Man Years:

Total:	2.366
Professional:	1.333
Other:	1.033

Objectives: To evaluate the role of the adrenal cortex in sodium retention; to investigate stimuli to aldosterone secretion

Methods Employed:

(1) A patient with aldosteronism, hypokalemic alkalosis and hypertrophy and hyperplasia of the juxta-glomerula apparatus was extensively studied with balance technic. (2) The effect of potassium given intravenously, intraarterially in the femoral artery, and intraarterially in the carotid arteries was measured in dogs before and after denervation of the carotid arteries with aldosterone secretion as endpoint. (3) The effect of caval constriction was measured before and after hypophysectomy with and without administration of hormones and with and without removal of the central nervous system above the pons. (4) The effect of caval constriction before and after nephrectomy was studied. (5) The effect of hypertensin, norepinephrin and renin was compared to that of ACTH.

Major Findings:

(1) It was found that the boy with hypertrophy of the juxta-glomerular apparatus secreted inappropriate amounts of aldosterone in almost all studies. The effect of expansion of the vascular volume with albumin was a very pronounced rise of aldosterone secretion in sharp contrast to the fall which is seen with secondary aldosteronism and the lack of effect in patients with aldosteroma and primary aldosteronism. (2) It was found that whereas potassium given intravenously at the rate of .6

mEq per minute did not give rise to increases of aldosterone secretion in the dog, the same dose given via the carotids consistently increased aldosterone secretion. The effect was the same before and after denervation of the carotid arteries. When the same dose was given into the femoral arteries there was slight but not significant increase in aldosterone secretion. An interesting finding in these studies was that potassium given intraarterially produces marked and reproducible increases in blood pressure. (3) Caval constriction was found to increase aldosterone secretion not only before but also after removal of the anterior pituitary. The effect was still present when the removal of the pituitary was accompanied by the removal of the central nervous system above the pons. It is apparent that there are stimuli to aldosterone secretion not dependent upon the pituitary or the suprapontine central nervous system. The effect of caval constriction was found to be markedly diminished or abolished by prior nephrectomy. In the nephrectomized hypophysectomized dog hypertensin gave rise to increases of aldosterone secretion. On occasion rises were also induced with norepinephrin and renin. Whereas norepinephrin does not appear to lead to increases of hydrocortisone secretion, the effect of hypertensin on aldosterone secretion is accompanied by an increase of hydrocortisone secretion.

Proposed Course of Project:

The effects of caval constriction in the nephrectomized hypophysectomized animal and in the animal with suprapontine brain removal are being studied in further detail and amplified. It is hoped to define further the factors which are necessary and functional in the normal control of aldosterone secretion.

Part B included Yes

Serial No. NHI-26
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Gann, D.S., Mills, I.H., Cruz, J.F., Casper, A.G.T. and Bartter, F.C. On the Mechanism of Decrease of Aldosterone Secretion in the Dog. Proc. Soc. Exper. Biol. and Med. 105: 158-161, 1960

Bartter, F.C., Mills, I.H., and Gann, D.S. Increase in Aldosterone Secretion by Carotid Artery Constriction in the Dog and its Prevention by Thyrocarotid Arterial Junction Denervation. J. Clin. Invest. 39: 1330-1336, 1960

Gann, D.S., Mills, I.H., and Bartter, F.C. On the hemodynamic parameter mediating increase in aldosterone secretion in the dog. Fed. Proc. 19: 605-610, 1960

Gann, D.S. and Bartter, F.C. Increase in aldosterone secretion by acute denervation of the thyrocarotid arterial junction. Am. J. Phys. 199: 193, 1960

Bartter, F.C. The Role of Hemodynamic Factors and of Potassium in the Control of Aldosterone Secretion. The First Intern. Congr. of Nephrology In press.

Pronove, P., MacCardle, R. and Bartter, F.C. Aldosteronism, Hypokalemia, and a Unique Renal Lesion in a Five Year Old Boy. First Intern. Congr. of Endoc. Copenhagen, 1960 Session IIIa, No. 84, pp.167-168.

Serial No. NHI-27
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3 Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Blood volume alterations in edematous states

Principal Investigators: Rahill, W.J., M.D., Thomas, J.P., M.D., Bartter, F.C., M.D., and Kliman, B., M.D.

Other Investigators: Delea, C.S. and staff

Man Years (calendar year 1960)
Total: 2.993
Professional: 1.923
Other: 1.070

Objectives:

Changes in blood volume in patients with edema will be correlated with changes in electrolyte balance, body water, and aldosterone secretion. Aldosterone secretion has been found elevated in some edematous patients. Whether blood volume consistently relates reciprocally to aldosterone secretion in these situations is unknown. Knowledge of the relation between blood volume and aldosterone secretion would help to clarify the mechanism of control of aldosterone in these edematous states.

Major Findings:

The technique for determining blood volume with I^{131} labelled albumin and Cr^{51} labelled red cells has been perfected. Studies in edematous patients have been carried out: patients with cirrhosis were studied before and after treatment with diuretics. Similar studies were done in patients with cardiac failure.

Incidental Findings of Investigators:

Patients with cardiac failure were shown to have a "leak" of albumin, so that I^{131} levels decline during the test, and render the test unreliable.

Proposed Course of Project:

The studies will be expanded to include normal subjects on

comparable regimens, and subjects with nephrosis.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Thomas, J.P., M.D. and Bartter, F.C., M.D.: On the relation between diuretic agents and aldosterone in cardiac and cirrhotic patients with sodium retention. Brit. Med. J. In press.

Thomas, J.P. and Bartter, F.C.: Blood volume measurements in normal subjects, patients with cirrhosis and cardiac disease. Clin. Sci. In press.

Bartter, F.C., M.D., Gann, D.S., M.D., and Thomas, J.P., M.D.: On the mechanisms of sodium retention in cardiac and hepatic disease. To be published as part of a book entitled "Conference on the Human Adrenal Cortex"--the proceedings of a conference held in Glasgow in July, 1960.

Serial No. NHI-28
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Action of Parathyroid Hormone

Principal Investigators: Bell, N.H., M.D. and Bartter, F.C., M.D.

Other Investigators: Delea, C.S., Smith, G., Diller, E.

Cooperating Personnel: Pronove, P., M.D., Children's Convalescent Hospital, Washington, D. C.

Man Years (calendar year 1960)
Total: .871
Professional: .284
Other: .587

Objectives:

1. To study the physiology of the parathyroid hormone
2. To clarify the diagnosis of hyperparathyroidism

Major Findings:

1. Tm of phosphorus: Maximal tubular reabsorption of phosphorus did not differ significantly between seven normals and seven patients with hyperparathyroidism, and was less than 2.0 mg. per min. in four patients. This portion of the project has been discontinued.

2. Phosphorus clearance: Phosphate clearance increased directly with phosphate intake and did not differ significantly between six normal subjects and twenty patients with hyperparathyroidism.

3. Tubular reabsorption of phosphorus: Per cent tubular reabsorption of phosphorus varied inversely with phosphorus intake and did not differ significantly between seven patients with hyperparathyroidism and six normal subjects.

4. Calcium infusion: Calcium infusion (15 mg./kg) showed a fall in urinary phosphorus greater than 21% on the day of infusion compared with the previous day and a "rebound" in urinary phosphorus greater than 12% the following day in six normal subjects. Eighteen patients with hyperparathyroidism showed either no fall with infusion or no "rebound" in urinary phosphorus following infusion of calcium.

5. Amphojel Test: A test of phosphorus deprivation was devised whereby patients received a low calcium, low phosphorus diet with aluminum hydroxide (amphojel) for ten days. During the test eighteen of eighteen patients with hyperparathyroidism and three normal subjects given parathyroid extract (100-200 units/day) developed hypercalciuria (urinary calcium greater than 250 mg. per day). Ten normal subjects and twenty-five patients with renal stones did not develop hypercalciuria (urinary calcium less than 250 mg. per day). This test, together with the calcium infusion test, has proved to be the most reliable in establishing the diagnosis of hyperparathyroidism.

Proposed Course of Project:

The calcium infusion test and the phosphorus deprivation test are still being evaluated.

Part B included Yes

Serial No. NHI-28

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Pronove, P., M.D., Bell, N.H., M.D., and Bartter, F.C., M.D.: The diagnosis of hyperparathyroidism. Metabolism. In press.

Pronove, P., M.D. and Bartter, F.C., M.D.: The production of hypercalciuria by phosphorus deprivation: a new clinical test for hyperparathyroidism. Metabolism. In press.

Part B

Honor, Awards, and Qualifications

Serial No. NHI-29
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Measurement of Aldosterone by Isotope Techniques

Principal Investigator: Kliman, B., M.D.

Other Investigators: None

Man Years (calendar year 1960)

Total:	.333
Professional:	.333
Other:	.000

Objectives:

The basic methods for assay of aldosterone by isotope dilution, and isotope derivatives labeled with carbon 14 and tritium have been used successfully. Further development is continuing in respect to purification methods, and changes in the usual isotope combinations.

Major Findings:

Attempts are being made to use gas phase chromatography for the separation and identification of aldosterone. The parent steroid and various specific derivatives have not been separable as yet, but such factors as temperature and column composition deserve further study. A sensitive detection method has been instituted using unlabeled steroid as a carrier for a mixture of the carbon 14 labeled internal standard and tritium labeled unknown aldosterone.

Proposed Course of Project:

This technique affords a triple identification and an isotope quantitation of aldosterone as a routine procedure, using chromatography on paper for purification. It appears likely that the processing time can be reduced and accuracy of the method increased, if gas phase chromatography can be applied.

Part B included No

Serial No. NHI-30
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Vitamin D

Principal Investigators: Bell, N.H., M.D. and Rose, G.A., M.D.

Other Investigators: None

Man Years (calendar year 1960):
Total: .111
Professional: .111
Other: .000

Objectives:

To determine the routes of excretion, breakdown products, and method of transport of vitamin D. Both H_3 and C_{14}-vitamin D_3 are being synthesized by Philips-Duphar, Weesp, Netherlands.

Major Findings:

Techniques have been developed for the chromatography of vitamin D and are available for studying the method of transport and metabolism in the rat.

Proposed Course of Project:

When radioactive vitamin D becomes available, the proposed studies will be initiated.

Part B included No

Serial No. NHI-31
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Calcium Metabolism in Sarcoidosis

Principal Investigators: Bell, N.H., M.D. and Bartter, F.C., M.D.

Other Investigators: Delea, C.S. and staff

Man Years (calendar year 1960):

Total:	.913
Professional:	.284
Other:	.629

Objectives:

To elucidate the mechanism of abnormal calcium absorption in patients with sarcoidosis, to clarify the role of vitamin D in the pathogenesis of the syndrome, and to determine the mechanism whereby steroids reverse or prevent the abnormal calcium absorption.

Major Findings:

Calcium, phosphorus and nitrogen balance studies have been carried out in four patients with sarcoidosis. Determinations of serum calcium, phosphorus, and vitamin D were obtained during the studies. Ca^{47} was given orally to measure calcium absorption.

The studies indicate that patients with sarcoidosis may have an abnormally low fecal calcium, that this may be associated with an abnormally elevated urinary and serum calcium, that prednisone may increase fecal calcium and may reduce serum and urinary calcium, that vitamin D may further reduce fecal calcium and result in greater hypercalcemia and hypercalciuria, and, finally, that this effect of vitamin D may be blocked or prevented by prednisone. Vitamin D levels in the serum increase but remain within the normal range during vitamin D administration, suggesting that the syndrome results from a hypersensitivity to the vitamin and not from hypervitaminosis D. Ca^{47} excretion following oral administration supports the calcium balance data.

Proposed Course of Project: Further data are being collected in preparation for publication of a manuscript.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Bell, Norman H., M.D., Gill, John R. Jr., M.D., and Bartter, F.C., M.D.: Calcium Metabolism in Sarcoidosis. A preliminary report of the findings is being published in the "Transactions of the International Conference on Sarcoidosis, Washington, D.C., June 3, 1960."

Serial No. NHI-32

1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title:

Water Loading in Normal Subjects and in Patients with Cardiac Failure

Principal Investigators: Bell, N.H., M.D. and Bartter, F.C., M.D.

Other Investigators: George Smith, E. Powell

Man Years (calendar year 1960):

Total:	.652
Professional:	.194
Other:	.458

Objectives:

To investigate the mechanism for the abnormally low free water clearance of patients with congestive heart failure. To determine the role of proximal tubular sodium reabsorption and antidiuretic hormone in producing this defect. To elucidate mechanisms involved by producing a comparable syndrome in normal subjects and in patients with diabetes insipidus by salt deprivation.

Major Findings:

Diuresis after oral and intravenous water loads:

The free water excretion in response to a water load by normal subjects has been shown to decrease progressively as salt depletion is intensified. An impaired diuresis of free water comparable to that shown by patients with decompensated cardiac failure is seen in the well hydrated normal subject after salt depletion.

Diuresis after intravenous loading:

Infusion of mixed isotonic solutions of mannitol (nonreabsorbable solute) and dextrose has been shown to increase free water clearance in both cardiacs and salt depleted normals above that seen with an equal load of dextrose alone. Both groups show a comparable degree of "correction" of the impaired diuretic response to intravenous dextrose when the mannitol mixture is used.

Serial No. NHI-32

No free water is formed when isotonic (5%) mannitol is infused into the normal non-salt depleted subject under these conditions. The decompensated cardiac and salt depleted normal do show positive free water clearance under these conditions.

Diuresis after sustained intravenous loading:

Substitution of mannitol for dextrose during a sustained water diuresis increases free water clearance in the decompensated cardiac and salt depleted normal but not in the non-salt depleted normal: evidence that the defect in free water clearance is due to an increase in proximal sodium reabsorption, thus preventing abstraction of sodium without water at more distal sites.

Diuresis following intravenous loading both with and without pitressin:

Diuretic response both with and without physiologic doses of exogenous pitressin (10 mU/kg/hr) have been shown to be quantitatively the same for normals and decompensated cardiacs, suggesting the water retention is not due to excessive endogenous antidiuretic hormone.

Proposed Course of Project:

Further data are being collected for publication as a paper.

Part B included No

Serial No. NHI-33
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS- NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effect of Amphotericin B on Renal Function

Principal Investigators: Bell, N.H., M.D.

Other Investigators: George Smith

Collaborating Units: Dr. Vincent Andriole, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases

Man Years (calendar year 1960):

Total:	.444
Professional:	.111
Other:	.333

Objectives: To investigate the mechanism for blood urea nitrogen elevation in patients with fungus infections receiving amphotericin B, an antifungal agent.

Major Findings:

Amphotericin B was found to markedly lower inulin (average fall 55 per cent) and para-aminohippurate (average fall 39 per cent) clearances in five patients receiving the drug (1591 to 3511 mg intravenously) over a period of 36 to 97 days. Both parameters improved after therapy, indicating that the renal damage is reversible.

Proposed Course of Project:

Further data are being collected in preparation for writing a manuscript.

Part B included No

Serial No. NHI-34 (c)
1. Gen. Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Unilateral Renovascular Disease.

Principal Investigator: Louis Gillespie, Jr., M.D.

Other Investigators: Thomas Stamey, M.D., Frank Spencer, M. D. (Johns Hopkins Hospital) and Louis Aviolé, M.D. (NCI).

Cooperating Units: Johns Hopkins Hospital and NCI.

Man Years:
Total: .20
Professional: .20
Other: 0

Patient Days: 800

Major Findings:

In last year's report we noted an increasing interest in evaluating those patients in our hypertensive population meeting necessary criteria for a renal or renovascular etiology of their hypertension. Accordingly, we have continued on an enlarging scale to study appropriate patients completely, performing operative correction when a positive diagnosis was made, and following these patients on a long term basis following surgery. The pertinent studies performed on such patients include renal arteriography, a modification of the Howard Test (retrograde ureteral catheterization and function studies) as developed by Dr. Stamey, and more recently performing the radioactive renogram as performed by Dr. Louis Aviolé of the National Cancer Institute. Also, we have responded to a request to participate in a continuing study section formed to gather and evaluate all data available on the diagnosis and treatment of renovascular hypertension. This NHI supported study section has met and preliminary plans are now established for its continuing function.

During the past year from a hospitalized hypertensive population approximating 80 in number, we have performed 20 renal arteriograms, 10 Howard Tests, and 40 radioactive renograms (all patients are undergoing this test for screening and control purposes). From these studies, a series of six cases have evolved with a diagnosis of renal

or renovascular disease thought to be contributing significantly to their hypertension. Operative correction, vascular repair in three cases and nephrectomy in three cases, has resulted in marked improvement or cure in all six cases. The duration of follow-up now ranges between five and 12 months.

The renal arteriogram is performed via arterial catheter inserted either through the femoral or brachial artery. The procedure is performed in cooperation with the radiology department and as such is not undergoing investigation. However, we are interested in the correlation of arteriographic findings with other studies and the clinical course of the patient, and in this series of six cases, arteriograms have been positive and have well delineated the vascular lesion in the five cases where it was performed. Follow-up arteriograms are planned where appropriate.

Ureteral catheterization with collection of urine from each kidney, measuring volume and performing function tests has been originally described as the Howard Test where volume and sodium concentration were the key measurements. Under the direction of Dr. Thomas Stamey various modifications have been made and applied to 10 patients on our service and found to be positive in all six of the above-mentioned cases. The modifications of the original Howard Test have been needed since the previous requirements of 50 percent reduced urine formation associated with 15 percent reduced sodium concentration on the affected side have not been found in all cases of unilateral renal disease, notably in cases of segmental infarction. The basis for Dr. Stamey's modification has been the observation in studies in dogs that the key pathophysiologic alteration in an "ischemic" kidney is the increased reabsorption of water. This occurrence will result in increased concentration of any osmotic substances measured on the affected side as opposed to the opposite kidney. Many substances have been measured including sodium and other electrolytes, urea, para-amino huppurate (PAH), inulin and creatinine. These substances have been measured also under various forms of diuresis since the type of induced diuresis has been shown to vary the disparity of various solutes being measured. At present the measurement of the concentration and clearance of PAH and inulin have been found to be quite sensitive indicators, showing the greatest degree of disparity in those cases of renal vascular disease studied, particularly when the test is performed under urea diuresis. In two cases a positive diagnosis would not have been made using sodium concentrations while the disparity of the PAH concentrations under water or urea diuresis varied between five and eight fold, reflecting the increased sensitivity of the latter measurements. Although the series here as well as cases similarly studied at Johns Hopkins remains small, to date there have been no false positive or negative results that we are aware of using this modification.

Once available in the Radiation Branch of the NCI, we began to perform the radioactive renogram on our entire hypertensive population,

and approximately 40 of these studies have been performed. This test consists of the intravenous infusion of a radioactive iodine tagged molecule which is rather selectively excreted via the kidneys. By performing columated counting of the radioactivity over each kidney simultaneously, significant differences in the amount and rate of excreted radioactivity may be detected. This is a simple procedure requiring evaluation for its sensitivity (i.e. how many false positive and false negative results) relative to the more difficult procedures already described. To date four cases with positive diagnoses of renovascular lesions have been studied with this technique, and all have demonstrated positive renograms. We plan to continue this evaluation of the renogram, leaning more heavily on it as a screening procedure as growth of our confidence in it permits.

Proposed Course of Project:

Continued evaluation of these diagnostic procedures, particularly the renogram is planned. Followup studies of patients who have undergone corrective surgery will be done. The possibility of beginning studies to pursue alterations in the metabolism of renin and angiotensin would require such a group of properly diagnosed patients with renovascular hypertension.

Part B included: Yes No

Serial No. NHI-35
1. Gen. Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Studies of the Cardiovascular Effects of Pharmacologic Agents and Amines in Experimental Animals and Man.

Principal Investigator: Leon I. Goldberg, M.D., Ph. D.

Other Investigators: Frances DaCosta (graduate student), J. Richard Crout, M.D. and T. Cooper, M.D. (NHI-Surg).

Cooperating Units: Heart Surgery

Man Years:
Total: 2.55
Professional: .95
Other: 1.60

Patient Days: 0

1. Effects of Monoamine Oxidase Inhibition on Ganglionic Transmission.

Studies completed in this laboratory have shown that administration of large doses of two monoamine oxidase (MAO) inhibitors, iproniazid and harmine, decreased transmission through the stellate ganglion of the dog and the superior cervical ganglion of the cat. Depression of ganglionic transmission was detected by observing that effects produced by pre-ganglionic stimulation (tachycardia in the dog; contraction of the nictitating membrane in the cat) were decreased after administration of the MAO inhibitors. Effects produced by postganglionic stimulation or by stimulation of the vagus (parasympathetic) nerve were not depressed. This data indicated that MAO inhibitors could exert a selective block of the sympathetic transmission and suggested that such a block could be related to the postural hypotension produced by the inhibitors in man.

Because many of the MAO inhibitors have direct effects on heart rate and on the nictitating membrane, a method was adopted for study of the electrical potentials transmitted through the ganglion, rather than the responses of end-organ. This method involved stimulation of the pre-ganglionic nerves of the superior cervical ganglion of the cat and recording from the post-ganglionic nerve. Additionally, this technic permits recordings from the pre-ganglionic nerve to determine that any block in transmission observed does not occur before the ganglion.

Preliminary studies have confirmed the fact that harmine and iproniazid do depress ganglionic transmission in the intact animal and additionally have shown that this block does not occur in the pre-ganglionic nerve. We have also demonstrated that several other inhibitors, MO-911 (N-benzyl-N-methyl-2-propynyl-amine·HCl), Monase (α-ethyltryptamine) and JB-516 (β-phenylisopropylhydrazine) also depress ganglionic transmission when given in large doses. Some of the agents, however, also depress parasympathetic function. Since the ganglionic depression produced by most of these drugs was transient and the block of MAO is considered to be irreversible, it is probable that the depression of ganglionic transmission is not related to MAO inhibition. One may only speculate that this transient block is related to the clinical effects of the inhibitors, but it is possible that only a slight modification of ganglionic function is needed to produce postural hypotension in man.

2. Pharmacological Studies of α-Methyl-Dopa and Other Decarboxylase Inhibitors in the Dog.

Intravenous infusion of 100-200 mg/kg of α-methyl-dopa (α-methyl-3,4-dihydroxy-DL-phenylalanine) to normotensive and renal hypertensive unanesthetised dogs produced sedation and lowering of the blood pressure (range 20-60 mm Hg). The reduction in pressure was primarily systolic and was greater in magnitude in the hypertensive animals. The hypotension did not begin for 2-3 hours and the peak effect was seen in 5-6 hours. In 24 hours the pressure had returned to pre-injection levels. In addition to hypotension transient sedation was also observed. In most experiments the animals appeared to be transiently sedated by the inhibitor but they were easily aroused by noise. These effects appeared to be similar to those observed after administration of the agent in man, thus affording an opportunity to study the mechanism of action of the agent by technics not possible in the human. In line with this concept, a relatively sensitive method of assaying decarboxylase activity in vivo was developed. L-dopa in doses of 10 to 20 mg/kg when administered intravenously to the anesthetised dog produced a pronounced and prolonged increment in heart contractile force. Since L-dopa increases contractile force due to its conversion to dopamine, this inotropic effect is a measure of decarboxylation. Alpha-methyl-dopa in doses of 100-200 mg/kg completely inhibited the effects of Dopa for 30 minutes and then the inhibitory effects gradually decreased so that the effects of Dopa had returned in 4-6 hours.

In addition to α-methyl-dopa, two other potent decarboxylase inhibitors, α-methyl-meta-tyrosine and α-methyl-2,3,-dihydroxyphenylalanine were studied in a similar manner. Each of these amino acids blocked the cardiac effects of L-dopa in doses of 100 mg/kg, as did α-methyl-dopa, but did not decrease blood pressure in the same normotensive and hypertensive dogs which had hypotensive responses to α-methyl-dopa. Because of this finding it appears that the hypotensive effects of α-methyl-dopa in the dog is not related to inhibition of decarboxylation. Therefore, screening of additional drugs for anti-hypertensive effects should not be made on the basis of inhibition of decarboxylation.

Further studies to ascertain the mechanism of action of α-methyl-dopa have been mostly negative. These studies have shown that the hypotension does not appear to be the result of ganglionic block or lack of responsiveness of end organs to norepinephrine or sympathomimetic amines such as tyramine. Similarly, norepinephrine depletion does not appear to be the reason for the hypotension since the heart responds to stimulation of sympathetic nerves (unlike after reserpine administration). Preliminary studies with a chronically implanted strain gauge arch have shown that there is a decrease in heart contractile force at the time of the hypotensive effect in the unanesthetised dog. The relationship of cardiac depression to the hypotensive effect is being evaluated.

3. Comparative Effects of Ritalin on the Inotropic Effects of Norepinephrine in the Intact Dog and in the Dog with Chronically Denervated Heart.

Previous studies in this laboratory have shown that Ritalin greatly potentiates the increments in heart contractile force produced by norepinephrine in the anesthetised dog (Fed. Proc. 18:396, 1959). Since similar potentiation occurs after chronic denervation of the heart, the present study was designed to ascertain whether Ritalin could further potentiate norepinephrine effects in the dog with chronically denervated heart. Therefore, the action of norepinephrine was determined before and after administration of Ritalin to seven such dogs (prepared by Dr. T. Cooper). In no animal with complete denervation was it possible to increase the norepinephrine response with Ritalin. On the other hand, there was varying degrees of potentiation in the animals who were not completely denervated, as determined by responses to stimulation of the stellate ganglion. Studies in collaboration with Dr. J. Richard Crout demonstrated that Ritalin does not deplete norepinephrine from the heart, nor does it interfere with the enzymes known to inactivate norepinephrine. Accordingly, it is postulated that the potentiation produced by Ritalin may be related to an effect on the binding of norepinephrine to non-receptor sites. A practical result of this study has been the use of Ritalin potentiation of norepinephrine as a sensitive guide of the adequacy of the denervation procedure.

4. Effects of Drugs on Myocardial Contractility in Man.

Collaboration has continued with the Surgery Branch and the Department of Anesthesia in the study of drug effects on myocardial contractility in patients undergoing cardiac surgery with cardiopulmonary bypass. In addition to the studies of digitalis and sympathomimetic amines, which were presented previously, a study of the inhalation anesthetic agent Fluothane has been completed. Seventeen patients were administered Fluothane in varying concentrations and in 15 of these depression of cardiac contractility was observed. The degree of depression was related to the concentration of the agent, suggesting that the lower concentrations would appear to be safer for patients with decreased myocardial reserve. Although a decrease in blood pressure usually reflected the decrease in cardiac contractility, in several patients

cardiac contractility remained depressed longer than the blood pressure. This information is important, for the anesthesiologist should be aware of the inadequacy of blood pressure recordings for evaluating cardiac depression produced by anesthetic agents.

Proposed Course of Project:

1. Further studies of the ganglionic blocking effect of monoamine oxidase inhibitors. Studies will be continued on effects of MAO inhibitors on ganglionic transmission. Other inhibitors will be studied and if possible results will be correlated with chemical determinations of norepinephrine and monoamine oxidase levels in the ganglia.

2. The pharmacological effects of α-methyl-dopa. A method is being devised with the cooperation of Dr. Samuel Fox to adopt the dye-dilution technic for determination of cardiac output for use in hypertensive dogs being treated with α-methyl-dopa. By use of simultaneous cardiac output and heart contractile force determinations, it is hoped that more information on the mechanism of action of α-methyl-dopa will be obtained. Other drugs similar to α-methyl-dopa (with and without decarboxylase inhibitory properties) will be studied for possible antihypertensive effects.

3. The direct effects of drugs on myocardial contractility in man will be continued in collaboration with the Clinic of Surgery. The next immediate objective is to compare the effects of several sympathomimetic amines on heart contractile force and peripheral resistance force during cardiopulmonary bypass.

Part B included: Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part B:

Publications:

1. Bloodwell, R. D., Goldberg, L. I., Braunwald, E., Gilbert, J. W., Ross, J. and Morrow, A. G. Myocardial contractility in man: The acute effects of digitalis, sympathomimetic amines, and anoxic cardiac arrest. Surg. Forum 10:532-535, 1960.

2. Goldberg, L. I., DaCosta, F. M. and Ozaki, M. Studies of the actions of the decarboxylase inhibitor, α-methyl-3,4-dihydroxy-phenylalanine, in the dog. Nature. 188:502-504, 1960.

3. Goldberg, L. I. and DaCosta, F. M. Selective depression of sympathetic transmission by intravenous administration of iproniazid and harmine. Proc. Soc. Exper. Biol. & Med. In Press.

4. Goldberg, L. I., Bloodwell, R. D., Braunwald, E. and Morrow, A. G. The direct effects of norepinephrine, epinephrine and methoxamine on myocardial contractile force in man. Circulation. In Press.

5. Bloodwell, R. D., Brown, E. C., Christensen, G. R., Goldberg, L. I. and Morrow, A. G. The effects of Fluothane on myocardial contractile force in man. Anesthesia and Analgesia. In Press.

6. Braunwald, E., Bloodwell, R. D., Goldberg, L. I. and Morrow, A. G. Studies on digitalis IV. Observations in man on the effects of digitalis preparations on the contractility of the non-failing heart and on total vascular resistance. J. Clin. Invest. In Press.

Serial No.: NHI-36 (c)
1. Gen. Med. & Exp. Therap.
2. Experimental Therapuetics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Studies on the Amine Pathways of Aromatic Amino Acid Metabolism in Man.

Principal Investigator: John A. Oates, M. D.

Other Investigators: Albert Sjoerdsma, M.D., Ph. D., J. Richard Crout, M. D., John Pisano, Ph. D.,(LCB), Herbert Weissbach, Ph. D., (LCB), Sidney Udenfriend, Ph. D., (LCB), Perola Zaltzman (Visiting Scientist) (LCB), E. B. Marsh, C. Muellenberg and M. Schroeder.

Cooperating Units: Laboratory of Clinical Biochemistry, NHI.

Man Years:
Total: 2.38
Professional: 1.30
Other: 1.08

Patient Days: 950

Major Findings:

1. Aromatic Amino Acid Decarboxylation.

Studies on the inhibition of aromatic amino acid decarboxylation in man by α-methyl-3,4-dihydroxy-DL-phenylalanine (αMD) have been extended. In the initial investigations, this agent was found to inhibit the decarboxylation of tyrosine and tryptophan in hypertensive patients, and in conjunction with this inhibition of amine biosynthesis, a reduction in the patient's blood pressure was observed. The anti-hypertensive action of αMD has been confirmed and it is now under evaluation as a possible therapeutic agent (see report of Dr. Gillespie). Since it was thought that tryptophan and tyrosine were being decarboxylated by the same enzyme catalyzing the decarboxylation of other aromatic amino acids, the effects of αMD or the decarboxylation of 5-hydroxy-tryptophan (5HTP), dihydroxyphenylalanine (DOPA) and phenylalanine were investigated.

Five-hydroxytryptophan decarboxylation was investigated initially by infusing this amino acid and measuring urinary excretion of the product, serotonin. To accomplish this, a more sensitive method for assay of urinary serotonin was developed, which employs separation of serotonin from urine by a weak cation exchange resin and assay of the

serotonin in the eluate fluorimetrically. It was found that αMD 2 gm. orally would reduce the excretion of serotonin following 5HTP infusion by about 60 percent. In a similar manner, the excretion of dopamine following infused Dopa was used an an index of Dopa decarboxylation. A 60 percent reduction of dopamine excretion was produced by αMD 2 gm. Dopa decarboxylation was investigated further by measuring excretion of the catecholamine metabolites, normetanephrine and methoxy-hydroxy-mandelic acid, in two patients with pheochromocytoma, both before and after αMD. Approximately 20 percent reduction in excretion of these catecholamine metabolites was observed following administration of αMD.

Phenylalanine decarboxylation was studied in a patient with phenylketonuria. Milligram quantities of phenylethylamine are excreted in the urine of phenylketonuric patients when a monoamine oxidase inhibitor is given, and can be measured semiquantitatively by paper chromatographic or paper electrophoretic methods after extraction from the urine. After an elevated phenylethylamine excretion was achieved with daily administration of a monoamine oxidase inhibitor, αMD was given as a 2.5 gm. single dose. This produced a reduction of phenylethylamine to less than 30 percent of the excretion on the monoamine oxidase alone, indicating that phenylalanine decarboxylation was also inhibited by αMD.

The inhibition of decarboxylation of all five of these amino acids by the competitive inhibitor, αMD, lends support to the concept that there is a general enzyme, L-aromatic amino acid decarboxylase, which will catalyze the decarboxylation of Dopa, tyrosine, phenylalanine, 5HTP, and tryptophan in man. Whether this enzyme may also play a significant role in histadine decarboxylation in man remains to be determined.

Since all the above studies were done with the racemic mixture, DL-αMD, it was desirable to find whether the total activity of the mixture on both human blood pressure and decarboxylation resided in the levo isomer. Studies on 5HTP decarboxylation in carcinoid patients demonstrated total lack of inhibitory activity by the dextro isomer, and studies on hypertensive patients demonstrated that the dextro isomer had little or no effect on either tyrosine decarboxylation or blood pressure, while the levo isomer proved effective as a hypotensive agent and as an inhibitor of tyrosine decarboxylase in one half the dosage required for the same effect by the racemic mixture.

Both in an effort to develop more effective therapeutic agents, and in attempting to determine whether decarboxylase inhibition is the mechanism of blood pressure reduction by αMD, studies on other decarboxylase inhibitors have been initiated. The first of these was α-methyl-meta-tyrosine, which as an *in vitro* inhibitor of decarboxylation is one-fourth to one-half as potent as αMD. Because of a less than 10 percent absorption of oral doses it has not been possible either to inhibit decarboxylation or to lower BP by oral administration of up to 6 gm. of this agent daily. Therefore, it would seem to offer little therapeutic

promise.

In order to facilitate evaluation of decarboxylase inhibition by these new agents, the method for urinary tyramine has been improved to increase its sensitivity.

2. The Carcinoid Syndrome:

Since CMD inhibited decarboxylation of infused 5HTP, it was of interest to see whether the same inhibition would occur in the tumor of patients with carcinoid syndrome. Because of the desirability of measuring substrate accumulation, a method for assay of urinary 5HTP was developed. This was accomplished by removing the serotonin from the urine by passage over a weak cation exchange resin and removal of CMD by passage over alumina. The effluent was then incubated with guinea pig kidney decarboxylase and the serotonin formed in this incubation was separated from the mixture on a weak cation exchange. Serotonin in the eluate was assayed fluorimetrically.

Using urinary excretion of the serotonin metabolite, 5-hydroxyindoleacetic acid (5HIAA) as an index of serotonin synthetis, it was possible to show that CMD produced a greater than 70 percent inhibition of serotonin synthesis in two patients with carcinoid syndrome. In both of these there were concommitant increments in 5HTP excretion indicating that the site of inhibition of serotonin synthesis was at the decarboxylation step. While the results in these two patients demonstrate the fact that it is possible to inhibit serotonin synthesis in a tissue such as carcinoid tumor, the hypotensive property of CMD has prevented administration of sufficient doses of the inhibitor to reduce serotonin synthesis in other patients, and definite therapeutic benefit was obtained with CMD in only three of eight carcinoid patients. It, therefore, seemed desirable to try other decarboxylase inhibitors in the carcinoid syndrome, in hope of finding one which would inhibit serotonin synthesis without lowering the blood pressure. Alpha-methyl-5-hydroxytryptophan (CM-5HTP) was selected for trial in patients after demonstrating that it almost completely inhibited serotonin synthesis in ascites mast cell tumors of mice. Because of the limited supply of this inhibitor, we have been able to use it in only one carcinoid patient to date. In that case, CM-5HTP 5.25 gm/day produced a greater reduction in serotonin aynthesis than did CMD 6.0 gm/day. This occurred despite the fact that CM-5HTP was shown to have been poorly (10%) absorbed from the intestinal tract in that patient. Neither inhibitor lowered his blood pressure. Further studies of CM-5HTP await synthesis of a new supply of this inhibitor.

One of our patients with carcinoid has proved to be an unusual variant of the syndrome. This 37 year old housewife with hepatic metastases arising from a gastric primary had a striking type of flush consisting of vivid red, sharply delineated patches with irregular borders. She was found to excrete chromatographically detectable amounts of 5HTP, serotonin and 5HIAA in the urine, whereas the usual

carcinoid excretes only 5HIAA in these quantities. Studies on infusion of 5HTP in non-carcinoid subjects shows that the proportions in which these 5-hydroxyindoles are excreted in this patient were almost identical to those proportions following 5HTP infusion, and suggest that the tumor is releasing 5HTP into the circulation instead of preformed serotonin. In addition to this apparent inability of the tumor to decarboxylate 5HTP, there was a greatly increased urinary histamine excretion.

The unusual features of this case were remarkably similar to those reported in a case of gastric carcinoid studied by Sandler as well as published and unpublished findings on a number of other gastric carcinoids, thus making it apparent that most of the metastatic gastric carcinoids constitute a unique variant of the carcinoid syndrome. Of practical interest was the remarkable clinical improvement produced by CMD in this patient, a therapeutic result which was superior to that obtained in the seven other carcinoids so treated.

3. Histamine:

The need to study histadine decarboxylation in man and the suspicion that the gastric carcinoid patient had an elevated histamine excretion prompted the development of a method of separating histamine from human urine to permit its fluorimetric assay.

Previously, urinary histamine has been measured by the cumbersome bioassay technique. Recently Dr. P. Shore (LCP) developed a sensitive method for measuring tissue histamine which employed the fluorescence produced when histamine is condensed with orthophthalaldehyde. However, unknown substances in the urine grossly interfered with measurement of urine histamine by the procedure as developed by Shore. Therefore, a new procedure for separation of histamine from urine (or other biologic material) was developed. This procedure depends upon the very high affinity of histamine for weak cation exchange resin at pH 6.5, which permits the differential elution of the column with appropriate buffers to remove interfering compounds. The histamine is then eluted and extracted, and assayed by formation of the orthophthalaldehyde fluorophor. Counter current distribution studies, the fluorescence spectras, and paper chromatography of the final extract have been done to establish the specificity of the method. The normal range in 15 subjects was found to be 23 to 102 μg/24 hours. The patient with gastric carcinoid excreted 545-580 μg/day.

4. Synephrine:

In studies with Dr. J. Pisano (LCB) the amine N-methyl-p-hydroxy-phenylethanolamine (synephrine) was identified for the first time in human urine. Identification procedures included color reactions on paper following 2-dimensional chromatography and electrophoresis,

fluorescent properties, identification of the p-hydroxy-benzaldehyde formed on periodate cleavage of the amine by ultraviolet absorption spectrum following separation on the gas chromatogram, and by identification of the methyl amine formed on periodate cleavage by paper chromatography. Following its identification, synephrine excretion was found to be increased by monoamine oxidase inhibition.

5. Studies Attempting to relate Platelet Adenosine Triphosphatase and Platelet Serotonin Uptake:

Because of the presumptive implication of adenosine triphosphatase (ATPase) in erythrocyte and nerve ionic transport, and because of properties in common between some ATPases and platelet serotonin uptake, a study of the properties of platelet ATPase has been undertaken. Platelets were found to be a very rich source of ATPase. Like serotonin uptake into platelets, the platelet ATPase was stimulated by potassium. However, unlike serotonin uptake, it is not possible to inhibit platelet ATPase with 10^{-5}M Reserpine. Obviously, no conclusions are possible at this preliminary stage of the investigation.

6. The Effect of Acetaldehyde on Amine Metabolism:

Previously, other investigators have shown metabolism of various amines to be altered by acetaldehyde or the combination of ethanol and disulfiram. It was postulated that an enzymatic inhibition was produced by the acetaldehyde. Though this postulate seemed dubious, the findings were of great interest in light of the cardiovascular effects of alcohol and disulfiram in man, which include hypotension.

Preliminary investigations with tryptamine and norepinephrine suggest that when these amines are incubated at pH 7.4 for 15 min at 37° with equimolar amounts of acetaldehyde that an amine-aldehyde complex (probably Shiff base) is formed which is chromatographically different from the amine itself and which lacks the pharmacologic activity of the amine. The pharmacologic significance of these findings in man remains to be explored.

Proposed Course of Project:

1. Aromatic amino acid decarboxylation: Studies will be conducted with intravenous α-methyl-meta-tyrosine and other decarboxylase inhibitors such as αM5HTP in an attempt to establish whether decarboxylase inhibition is the mechanism by which αMD lowers B.P.

2. Carcinoid syndrome: The effect of decarboxylase inhibitors such as αM5HTP on serotonin synthesis will be investigated further. If this compound proves to be a more potent inhibitor of serotonin synthesis than αMD, the possibility that αM5HTP might act in part by inhibiting tryptophan hydroxylation should also be studied. In addition, the therapeutic use of these inhibitors will be evaluated.

3. Histamine: Since elevated amounts of histamine are excreted in a carcinoid patient whose tumor releases 5HTP, the possible release of histamine by circulating 5HTP will be investigated. It is also hoped that by establishing the amount of circulating histamine required for a significant increase in urinary histamine, it will be possible to better understand the applicability of measuring urine histamine as an index of histamine release or synthesis in man. Histadine infusions or the use of a diamine oxidase inhibitor may provide tools by which histadine decarboxylation may be investigated in man.

The relative ease of the fluorimetric assay of urinary histamine should allow us to study the possible role of histamine in pathologic and physiologic states with more facility.

4. Synephrine: Synephrine and norsynephrine are probably synthesized from tyramine by the same side chain β-oxidase which converts dopamine to norepinephrine. Therefore, it may prove feasible to study this interesting enzyme in man by measuring the conversion of tyramine to synephrine and norsynephrine and their acid metabolite p-hydroxy mandelic acid. Also, C_{14} tracer studies of metabolism of tyramine along this route may shed light on these pathways.

5, ATPase: Studies of the properties of platelet ATPase are being continued, in search of possible relationships between this enzyme and amine transport.

6. Acetaldehyde: The preliminary observations suggesting that an amine-acetaldehyde complexing may be an inactivation mechanism must be confirmed and extended in vivo.

Part B included: Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part B.

Publications:

1. Oates, J. A., Gillespie, L., Udenfriend, S. and Sjoerdsma, A. Decarboxylase inhibition and blood pressure reduction by α-methyl-3,4-dihydroxy-DL-phenylalanine. Science 131: 1890-1891, Jan. 1960.

2. Oates, J. A. Measurement of urinary tryptamine, tyramine and serotonin: Application in studies of monoamine oxidase and amino acid decarboxylase inhibitors in man. Methods of Medical Research, Vol. 9, In Press.

3. Sjoerdsma, A., Oates, J. A., Zaltzman, P. and Udenfriend, S. Serotonin synthesis in carcinoid patients: Its inhibition by α-methyl-dopa, with measurement of associated increases in urinary 5-hydroxytryptophan. New Eng. J. of Med. 263:585-588 Sept. 1960.

4. Pisano, J. J., Oates, J. A., Karmen, A., Sjoerdsma, A. and Udenfriend, S. Identification of N-methyl-p-hydroxyphenylethanolamine (synephrine) in human urine. J. Biol. Chem. In Press.

5. Oates, J. A. and Sjoerdsma, A. Neurological effects of tryptophan in patients receiving a monoamine oxidase inhibitor. Neurology. In press.

Serial No. NHI-37 (c)
1. Gen. Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Clinical Investigation of Actions and Metabolism of Cardiovascular Drugs.

Principal Investigator: Louis Gillespie, Jr., M.D.

Other Investigators: David Horwitz, M.D., John A. Oates, M.D., Albert Sjoerdsma, M.D., Ph. D., Irene Tamagna, M.D. (GW) and Harold Orvis, M.D. (GW).

Cooperating Units: George Washington University Hospital

Man Years:
Total:
Professional:
Other:

Patient Days: 4800

Major Findings:

1. Drug Therapy in Hypertension.

a) Monoamine Oxidase Inhibitors: Studies begun three years earlier into the antihypertensive properties of the monoamine oxidase inhibitors have continued but also have taken a somewhat different turn. Hepatic toxicity observed with iproniazid (Marsilid), several analogues of iproniazid, as well as Catron (JB-516) coupled with the previously reported optic nerve toxicity observed with Catron (JB-516) and isonicotinic acid hydrazide has caused us to be concerned that a relationship might exist between the hydrazide structure common to the above mentioned inhibitors and hepatic and optic toxicity. Therefore, we have decided to pursue active investigation of only those amine oxidase inhibitors whose structure does not contain the hydrazine moiety. One such promising agent, N-benzyl-N-methyl-2-propynyl-amine HCl, MO-911 has been studied in hypertensive patients for both its amine oxidase inhibiting potency as well as antihypertensive properties. Promising preliminary results have been obtained and this agent is now being studied in our outpatient group and in a larger similar study in the Hypertension Clinic of George Washington Hospital under the supervision of Dr. Horwitz and Dr. Irene Tamagna. To date there has been no evidence of clinical toxicity.

We have continued to observe a correlation between effective enzyme inhibition with various inhibitors and orthostatic lowering of blood pressure. It is hoped that further understanding of the mechanism of sympathetic block will result from current studies in animals as reported in another project summary.

b) Guanethidine (Ismelin): We have continued to evaluate this agent in hypertensive patients on an outpatient basis during the past year. We have administered the drug to 15 clinic patients for periods of from one to nine months. We have found this drug to be an effective antihypertensive agent, blocking only the sympathetic portion of the autonomic nervous system and producing predominantly an orthostatic response. This agent is free of those side effects representative of parasympathetic blockade, and indeed its most common side effect appears to be diarrhea, presumably the result of an intestinal tract responding to overbalanced parasympathetic stimulation. Our results are consistent with those reported by other investigators. The hypotensive response obtained with this agent has also been used for comparative purposes in evaluating other enzyme-inhibiting agents more pertinent to our immediate program.

c) α-Methyl-dihydroxyphenylalanine (α-methyl-dopa): Since the last annual report we have completed evaluation of this drug in the treatment of hospitalized hypertensive patients, finding a hypotensive response occurring in each of the first ten patients studied. Furthermore, the hypotensive response occurred within 24 to 36 hours of beginning administration of the agent and ceased within a similar time period following discontinuation of administration. Notably, a recumbent as well as orthostatic effect was observed in those patients having mild to moderate hypertension. In addition to this study group another 15 hospitalized patients with diagnoses of essential hypertension or carcinoid syndrome have received α-methyl-dopa. Two cases of drug induced fever were observed, one of which was associated with transient abnormalities of liver function. Effects other than hypotension observed included an initial period of drowsiness lasting 24 to 48 hours and then receding to a point of permitting the patient to remain alert and not sleepy though in many cases more tranquil. Additionally, all patients have slept well at night in contrast to the often observed insomnia seen with amine oxidase inhibitors.

Studies with α-methyl-dopa have been extended to 12 clinic patients receiving the drug for periods of 3 to 15 months who were studied on this agent alone, in combination with chlorothiazide, in comparison to chlorothiazide alone, and where permitted by the degree of hypertension in a double placebo period. Preliminary evaluation of these results

indicate that α-methyl-dopa is an effective antihypertensive agent, more potent than chlorothiazide alone, and its effect is enhanced by combination with the thiazide. During this study we have not observed any further hepatic toxicity. Three patients of the entire group to receive this compound (both in and out of the hospital) have responded with acute depressive reactions which subsided directly without complication upon discontinuing the drug. It is of interest that two of these three patients had previously experienced similar reactions when receiving Rauwolfia derivatives. In the clinic group no other undesirable effects have been observed other than an occasional delay of ejaculation in male subjects. The tranquil effects accompanied by sound nocturnal sleep appears to be a desirable alternate effect of α-methyl-dopa therapy.

Resolution of the racemic form of this compound into its dextro and levo rotatory isomers permitted studies in patients with each isomer. We have found that only the levo-rotatory form possesses antihypertensive (and also enzyme-inhibiting) properties, and this has afforded a reduction of total dosage by half without sacrifice of effect. Currently, outpatients are receiving the levo form exclusively in extended clinical trials.

Most recently, we have turned to the use of α-methyl-dopa intravenously to determine its possible efficacy in the treatment of accelerated or malignant hypertension with encephalopathy. Preliminary results show that a brief infusion of 500 to 1000 mg. of α-methyl-dopa results in a significant hypotensive response beginning within one hour, maximum at 4 hours, waning at 12 hours. Further studies in more patients will be necessary to bear out an initial impression that this agent may be useful in treating this type of medical emergency.

Concomitant biochemical studies have been performed in many of the hospitalized patients receiving this agent orally or parenterally to demonstrate that α-methyl-dopa is an effective inhibitor of the enzyme L-aromatic amino acid decarboxylase, the enzyme which catalyzes the formation of several vasoactive amines from precursor amino acids. The results of these studies show clearly an enzyme-inhibiting affect and are discussed more fully elsewhere.

Proposed Course of Project:

a) Continuation of drug evaluation: With reference to α-methyl-dopa, we are continuing to evaluate the levo form of this drug in clinic patients, paying particular attention to any long-term toxicity which may develop as well as the possible development of tolerance to

this drug. Further studies concerned with the hypotensive response to intravenous administration of this drug is already in progress. These studies are so designed to measure biochemical alterations at the same time. Other analogues of α-methyl-dopa are also becoming available. Investigation of such drugs regarding their biochemical potential as well as hypotensive properties is projected; absorption of these compounds will also be studied by comparing oral and intravenous results.

b) Hemodynamics of enzyme inhibitors: Because of the unique biochemical and pharmacologic properties of these agents, it is of considerable importance to study the hemodynamic responses resulting from administration of these compounds. This is a difficult procedure since one of the important determinants, the cardiac output, requires removal of moderate amounts of blood from the vascular space, which in itself alters dynamics. However, it is hoped that appropriate precautions will permit determination of various measurements of cardiac and peripheral vascular functions. Such studies might elucidate whether these compounds were causing hypotension because of direct cardiac effect, peripheral vasodilatation, etc.

Part B included Yes No

Part B.

Publications:

1. Sjoerdsma, A., Oates, J. A. and Gillespie, L., Jr. Quantitation of monoamine oxidase inhibition produced with various drugs in man. Proc. Soc. Exper. Biol. & Med. 103:485-487, 1960.

2. Gillespie, L., Jr. Clinical pharmacology of newer antihypertensive agents, monoamine oxidase and decarboxylase inhibitors, bretylium tosylate and guanethidine. Annals of the New York Academy of Sciences 88:1011-1019, 1960.

3. Sjoerdsma, A. Newer biochemical approaches to the treatment of hypertension. Annals of the New York Academy of Sciences 88: 933-938, 1960.

4. Gillespie, L., Jr. and Sjoerdsma, A. Monoamine oxidase and decarboxylase inhibitors as antihypertensive agents. Med. Clin. N. Am. 1960. In Press.

5. Gillespie, Louis, Jr. Some new drugs in the treatment of hypertension. Military Medicine. In Press.

6. Sjoerdsma, A. Relationships between alterations in amine metabolism and blood pressure. Circulation Res. In Press.

Serial No. NHI-38 (c)
1. General Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Metabolism of Hydroxyproline and Collagen.

Principal Investigator: Darwin Prockop, M.D.

Other Investigators: Sjoerdsma, A., M.D., Ph.D. and Keiser, H., M.D.

Man Years:
Total: 1.25
Professional: 1.25
Other: 0

Patient Days: 1000

Major Findings:

1. With the development of improved methods for the analysis of hydroxyproline (HPr) a series of studies over the past two years have been conducted on the significance of urinary HPr excretion in man. These studies have established that urinary HPr arises primarily from the breakdown of body collagen, and that the amount excreted is a useful index of collagen synthesis and degradation. Preliminary measurements indicate that there is a progressive decrease in the amount of HPr excreted in successive decades of life, and that urinary HPr excretion may reflect the "metabolic age" of an individual's collagen.

2. Experiments in which large amounts of gelatin were fed to patients and to normal controls showed that the amount of urinary HPr peptides is increased by feeding HPr in the form of a protein. This appears to be one of the first demonstrations that complete peptides can be absorbed from the gastro-intestinal tract. A recent modification of the HPr method has enabled us to measure HPr peptides in plasma. Studies now in progress indicate that the total amounts of HPr peptides absorbed after the feeding of gelatin is small, but that the kidney is unable to retain these peptides, and they are rapidly cleared into the urine.

3. In addition to the other techniques, we have developed a simple technique for measuring the specific activity of radioactively labelled HPr. This has led to measurements of the turnover of various forms of collagen in the rat. Contrary to earlier beliefs, it has been demonstrated that although body collagen, taken as a whole, is metabol-

ically inert, there are forms of collagen which are both synthesized and degraded rapidly.

4. The availability of a simple method for HPr specific activity has also enabled us to study the synthesis of collagen. In vivo and in vitro studies in the chick embryo have established that collagen is synthesized by the same microsomal pathways suggested for the synthesis of other proteins.

Proposed Course of Project:

1. Further studies on changes in HPr excretion with age should establish that the amount excreted is directly related to the metabolic age of an individual's collagen. Since reports in the literature suggest a relationship between the age of collagen and its solubility, it may be possible to relate the amount of HPr excreted to the solubility of collagen in biopsy material from human subjects. These measurements should provide valuable tools in the study of hereditary and acquired diseases which affect connective tissue. At the moment we are particularly interested in Marfan's syndrome, in progeria and pseudoxanthoma elasticum.

2. Studies are underway on the absorption, metabolism, and renal excretion of HPr peptides. We hope to extend this to include the fate of other peptides in man.

3. We will undertake further radioactive studies in rats to determine the size of various collagen pools and to define their metabolic interrelationships.

4. The chick embryo systems for the synthesis of collagen will be developed further so that the intermediate steps in collagen synthesis, particularly the hydroxylation of proline, can be defined. Attempts will also be made to use these systems to measure the effects of various drugs on the synthesis and breakdown of collagen.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part B.

Publications:

1. Prockop, D. J. and Udenfriend, S. A specific method for the measurement of hydroxyproline in urine and tissues. Analyt. Biochem. In Press.

Serial No. NHI-39
1. General Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Inhibitors of the Fibrinolytic System.

Principal Investigator: A. Sjoerdsma, M.D., Ph.D.

Other Investigators: J. T. Correll, Ph.D. (A guest worker).

Man Years:
Total: .65
Professional: .65
Other: 0

Patient Days: 0

Major Findings:

1. It is not established whether excess deposition of fibrin in clinical pathologic states is due to a deficiency of fibrinolysin or an excess of inhibitor activity. Attempts to solve the problem have been inconclusive (Physiol. Rev. 39: 343, 1959). As a continuation of studies initiated with Drs. I. M. Nilsson and J. Waldenstrom, Malmo, Sweden, an attempt is being made to ascertain the role of inhibitor activity. Exploratory studies have recently been completed towards developing a method which might prove suitable for measuring the inhibition of fibrinolysis.

2. Initial investigations confirmed that clotted human plasma previously treated with streptokinase (SK) would lyse. It was also found that the euglobulin precipitate (pH 5.3, low ionic concentration) from a given volume of human plasma, dissolved in saline, treated with SK and clotted, yielded a clot more readily lysed than the equivalent of whole plasma similarly treated. Subsequent studies revealed that the supernatant was inhibitory to clot lysis. Much of the inhibition, however, was due to the presence of SK antibody. This made the use of SK activated lytic systems impractical for assay of fibrinolysis inhibitor (s) in plasmas.

Urokinase will also initiate fibrinolysis (Proc. Soc. Exper. Biol. and Med. 81: 675, 1952); no antibodies have been reported. Hence, attempts were made to develop a method employing urokinase. With a limited supply of the enzyme it was found that a system composed of 0.1 ml of 5% human fibrinogen, 0.1 ml (1 unit) of urokinase (Mich. St. Health Labs.), 0.7 ml of veronal-saline (pH7.3), and 0.1 ml of bovine thrombin in 3% $CaCl_2$, demonstrated clot lysis in around 16 minutes. When human

plasma supernatant (inhibitor fraction?) was added in graduated volumes at the expense of saline, clot lysis was prolonged (6 to 47 min.) in proportion to the amount of supernatant added. The results suggest a possible lysis-inhibitor technique.

Proposed Course of Project:

When an adequate supply of urokinase is received it is proposed to establish the lysis time of a standardized human fibrinogen-urokinase system; to quantitate the inhibition of such a system with epsilon amino caproic acid (e-ACA); and to ascertain the inhibitory activity of "normal" human plasmas in terms of e-ACA.

With the establishment of a technique and within its limitations, it is planned to study fibrinolysis inhibition in a variety of clinical pathologic states; investigate drug (insulin substitutes, insulin, heparin, cholesterol blockers, etc.) influence both in vivo and in vitro; study animals in shock, germ free animals, etc. A search for anti-inhibitor materials might be made.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part B:

Publications:

1. Sjoerdsma, A. and Nilsson, I. M. Aliphatic amino compounds as inhibitors of plasminogen activation. Proc. Soc. Exp. Biol. and Med. 103:533-535, 1960.

2. Nilsson, I. M., Sjoerdsma, A., and Waldenstrom, J. Anti-fibrinolytic activity and metabolism of E-aminocaproic acid in man. Lancet, 1322-1326, 1960.

3. Sjoerdsma, A. and Hanson, A. Determination of E-aminocaproic acid in urine by means of high voltage paper electrophoresis. Acta Chim. Scandinav. 13:2150-2151, 1960.

Serial No. NHI-40 (c)
1. Gen. Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Studies on Vasoactive Substances

Principal Investigator: Albert Sjoerdsma, M.D., Ph. D.

Other Investigators: J. R. Crout, M.D., Louis Gillespie, Jr., M.D. J. A. Oates, M.D., Sidney Udenfriend, Ph. D. (LCB), John Pisano, Ph. D. (LCB), Robert Creveling (graduate student, LCB), Robert Levine, M.D., W. Lovenberg, Consuelo Muellenberg and Doris Watts.

Cooperating Units: Laboratory of Clinical Biochemistry.

Man Years:
Total: 2.55
Professional: .90
Other: 1.55

Patient Days: 500

Major Findings:

1. Diagnosis of Pheochromocytoma.

Using improved methods, urinary catecholamines, the methoxy-amine metabolites (metanephrine and normetanephrine) and 3-methoxy-4-hydroxy-mandelic acid (MOMA) were measured in 23 cases of pheochromocytoma and in a large group of patients with essential hypertension. The upper limit of control values for each assay was 0.1 mg/day, 1.3 mg/day and 6.0 mg/day respectively. A diagnostic elevation of all three indices was found in 20 of the 23 cases; the catecholamine assay was diagnostic in all cases, the single test giving the largest relative increase over normal was either the assay of catecholamines or the methoxy-amines. Measurement of the latter compounds affords a rather simple means of screening hypertensive patients for pheochromocytoma. In the course of these studies it was found that the production of catecholamines as indicated by urinary products is not elevated above normal in essential hypertension.

2. Catecholamine Metabolism.

Previous studies by others have shown that norepinephrine is metabolized largely by two alternate enzymes: catechol-O-methyl-

transferase and monoamine oxidase. The present study was undertaken to evaluate which of these enzymes is of greater significance in the metabolism of norepinephrine in the brain and heart of the rat.

Monoamine oxidase activity, measured in vitro, was found to be 3.6 and 5 times greater than catechol-O-methyl transferase activity in the brain and heart respectively. However, in rat liver catechol-O-methyl transferase activity was 15 times greater than monoamine oxidase activity.

A significant increase in the catecholamine content of rat brain and heart was produced by the intraperitoneal administration of four different monoamine oxidase inhibitors. The intraperitoneal injection of pyrogallol, an inhibitor of catechol-O-methyl transferase, failed to increase the catecholamine content of the brain or heart, even after 18 hours of enzyme inhibition.

Norepinephrine was injected intravenously into rats, and the rate of disappearance of the amine from the plasma and heart was determined. In animals pre-treated with iproniazid the major finding was a marked accumulation of amine in the myocardium, relative to the concentration present in plasma; clearance of norepinephrine from the plasma was only moderately prolonged. Rats pre-treated with pyrogallol, on the other hand, demonstrated a severe impairment in their ability to metabolize circulating norepinephrine.

These experiments show that endogenous norepinephrine accumulates in the brain and heart of the rat when monoamine oxidase is inhibited; exogenous norepinephrine accumulates in the heart under the same conditions. Neither of these effects is produced by a catechol-O-methyl transferase inhibitor. The results suggest that oxidative deamination is the more significant pathway in the metabolism of NE in the brain and heart of the rat. This conclusion is quite compatible with the observation that O-methylation is the major pathway for circulating norepinephrine if one assumes that the liver participates in the metabolism of circulating amine. The liver is by far the richest source of COMT in the rat, and O-methylating activity in this organ greatly exceeds that of monoamine oxidase.

3. Monoamine Oxidase (MAO) Inhibition.

Urinary tryptamine and tyramine continue to afford an excellent index of this in man. Correlation between biochemical and hypotensive effects was found again in studies with a new, non-hydrazine inhibitor, N-methyl-N-benzyl-2-propynyl-amine·HCl (MO-911). Urine from patients receiving MAO inhibitors has provided a rich biological source of amines, many of which are still unidentified. The most recently discovered naturally occurring amines from this source are synephrine and norsynephrine. These compounds differ from epinephrine and norepinephrine only in lacking a meta-hydroxyl group. These structural similarities

indicate a parallel in biosynthetic pathways which may have physiologic implications. The role of tyramine as a precursor of the synephrines in man is currently under study.

Recently, a new procedure for measuring monoamine oxidase activity in tissue was developed. This was needed for accurate assays in organs with weak enzyme activity (e.g. dog heart) or in situations where only small amounts of tissue are available (e.g. sympathetic ganglia, needle biopsies, etc.). The method involves assay of indoleacetic acid (IAA) formed during incubation with tryptamine. While only a small percentage of the substrate is oxidized, an increment in indoleacetic acid can be easily measured since blank values are negligible and the fluorimetric assay of IAA is highly sensitive.

4. Inhibition of Aromatic Amino Acid Decarboxylation.

Alpha-methyl-dopa has received extensive chemical and clinical evaluation in man (see reports by Oates and Gillespie). The blood pressure actions are currently considered to be mediated via a depletion and/or diminished release of norepinephrine in the sympathetic nervous system. This is leading to attempts to determine the status of sympathetic nerve norepinephrine in man as indicated by alterations in urinary catecholamine metabolites after treatment with reserpine, α-methyl-dopa, guanethidine, ganglion-blocking agents and certain sympathomimetic amines, alone or in combination.

Proposed Course of Project:

Interest in new cases of pheochromocytoma will be maintained though no new research studies are planned for the moment. Methods for measuring the methoxy-amines and MOMA will be improved to permit more accurate assay of normal levels. Continued identification of urinary amines in MAO-blocked patients will be pursued - use of gas chromatography may speed the problem. Relationships of MAO and decarboxylase inhibition will be further clarified by additional inhibitors currently under development. As mentioned, attempts will be made to evaluate in more detail the status of sympathetic nerve norepinephrine in hypertensives, untreated and treated.

Part B included: Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part B.

Publications:

1. Crout, J. R. and Sjoerdsma, A. Catecholamines in the localization of pheochromocytoma. Circulation 22:516-525, 1960.

2. Crout, J. R. Determination of catecholamines in urine. Standard Methods of the American Association of Clinical Chemists. In Press.

3. Crout, J. R., Pisano, J. J. and Sjoerdsma, A. Urinary excretion of catecholamines and their metabolites in pheochromocytoma. Am. Ht. J. In Press.

Honors:

Dr. Albert Sjoerdsma nominated to Council of High Blood Pressure Research, American Heart Association, November, 1960.

Serial No. NHI-41 (c)
1. General Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Hemodynamic and Gastrointestinal Effects of Certain Drugs in Man.

Principal Investigator: David Horwitz, M. D.

Other Investigators: Leon I. Goldberg, M.D., Albert Sjoerdsma, M.D., Ph.D., Louis Gillespie, Jr., M.D., and Samuel M. Fox, III, M.D.

Man Years:
Total: .88
Professional: .88
Other: 0

Patient Days: 2000

Major Findings:

1. Monoamine Oxidase Inhibitors.

a. Use in Hypertension: Study of monoamine oxidase (MAO) inhibitors in hypertension has continued in the present year. Because of the limited usefulness of hydrazine containing MAO inhibitors attributable to low potency or toxicity, emphasis was placed on the evaluation of an inhibitor of new structural configuration. The non-hydrazine MAO inhibitor, N-benzyl-N-methyl-2-propynyl-amine·HCl (MO-911) received its initial trial in a group of nine patients. It was found that the drug effectively lowered standing blood pressure, was a potent MAO inhibitor of long duration, and produced a minimum of undesirable side-effects.

A study of the chronic effects of this drug has been launched, in collaboration with Dr. Irene Tamagna and Dr. Harold Orvis of the George Washington Hospital Hypertension Clinic. The study group will approximate 30 patients followed for six months while receiving the drug, with control placebo periods before and after treatment. Judgement of the potential usefulness of MO-911 must await completion of such long-term studies.

Previous observations in this Section have shown that a variety of MAO inhibitors share the property of lowering blood pressure. With the exception of trans-DL-2-phenylcyclopropylamine (SKF-385) all were hydrazine derivatives, however. The finding that this new type of MAO

inhibitor also has hypotensive properties lends support to the hypothesis that pronounced MAO inhibition produces orthostatic blood pressure reduction.

b. Use in Angina: Although MAO inhibitors have been reported to be useful in the treatment of angina pectoris the mechanism of this effect has not been defined. Repeated observations in two patients receiving the MAO inhibitor, isocarboxazid, have revealed obtunded rises in pulse rate and blood pressure in response to exercise. These findings are presumptive of a restricted increase in cardiac work in response to exercise and may explain the reported beneficial effects of MAO inhibitors in patients with angina pectoris. These observations will be repeated in other subjects and will be supplemented by direct measurement of cardiac output by the indicator-dilution technique.

2. Effects of Dopamine in Man.

Study of the cardiovascular effects of dopamine has been completed. Dye dilution outputs in collaboration with Dr. Samuel M. Fox, III have been performed in seven normal controls and three patients. They confirm the initial findings that dopamine has the predominant effect of increasing cardiac output while causing a negligible effect on heart rate, a moderate fall in peripheral resistance and an increase in systolic arterial pressure with little effect on diastolic pressure. Increases in oxygen consumption are minor and increases in blood glucose levels are slight, but consistent, at hemodynamically-effective doses.

3. Modification of the Stimulatory Effect of 5-Hydroxytryptophan (5HTP) on Bowel Motility by α-Methyl-Dopa (αMD) in Man.

Five-HTP, upon decarboxylation, is converted to serotonin which is capable of provoking intestinal hypermotility. In an attempt to evaluate whether αMD in doses used in man is a physiologically effective decarboxylase inhibitor, the response of small intestinal motility to 5HTP infusions was evaluated in two subjects before and during αMD therapy. One subject showed abolition of the 5HTP response and the other showed a diminished response.

4. Modification of Pressor Responses to Tyramine by Drugs.

The pressor response to tyramine has been abolished by large doses of reserpine in animals and has been restored following norepinephrine infusion. It has been proposed that tyramine acts by releasing norepinephrine from sympathetic nerves and failure of tyramine response has been suggested as a measure of norepinephrine depletion. In one subject it was found that conventional doses of norepinephrine (0.25 mgm daily) over a three week period sufficed to abolish the response to a standard dose of tyramine. Similar studies are underway with αMD and will be pursued further with reserpine.

Proposed Course of Project:

1. Study of the chronic effects of the MAO inhibitor, MO-911.

2. Further studies of the influence of α-methyl-dopa on the response of the small intestine to 5HTP infusions will be carried out.

3. The effects of sympatholytic agents on the response to exercise will be studied with particular emphasis on the effects of MAO inhibitors in angina.

4. Study of amine release by reserpine and α-methyl-dopa will be continued with measurement of urinary amines and metabolites directly and correlation of these findings with the results of tyramine infusions.

Part B included: Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1960

Part B:

Publications:

1. Horwitz, David, Goldberg, Leon I., and Sjoerdsma, Albert. Increased blood pressure responses to dopamine and norepinephrine produced by monoamine oxidase inhibitors in man. J. Lab. and Clin. Med. 56:747-753, 1960.

Serial No. NHI-42 (c)
1. General Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Responses of Blood Unesterified Fatty Acid Levels: A Phase of Studies on Vasoactive Substances.

Principal Investigator: David Horwitz, M.D.

Other Investigators: Peter Mueller, M.D. (N.C.I.) Daniel Steinberg, M.D., Ph.D. (LCPM) and Eugene Feigelson, M.D. (LCPM).

Cooperating Units: National Cancer Institute and Laboratory of Cellular Physiology and Metabolism.

Man Years:
Total: .40
Professional: .40
Other: 0

Patient Days: 250

Major Findings:

1. Our interests have centered on the blood unesterified fatty acid (UFA) response as a non-vascular measure of reactivity to sympathominetic amines and as a factor in atherogenesis. Studies a-d were performed in collaboration with Dr. Peter Mueller and study e in collaboration with Drs. Daniel Steinberg and Eugene Feigelson.

a) UFA responses to norepinephrine in hypertensives: Hypertensives show an enhanced pressor response to doses of epinephrine and norepinephrine, in comparison with normotensives. It is not established whether this results from differences in blood vessels, receptors or the pattern of amine degradation. It was hoped that study of a non-vascular response, the UFA response to intravenous norepinephrine might help to define this. Thirteen observations were made in 3 subjects. In view of the variability of response and the lack of a reproducible dose-response relationship in the same subject comparison of UFA responsiveness to norepinephrine administration between groups of

subjects did not appear promising.

b) UFA responses to cold pressor test in hypertensives: Hypertensives show an increased incidence of arteriosclerotic changes. The hypothesis that this might result from differences in fat metabolism related to increased UFA responsiveness was considered. As an approach to this problem the UFA response to a standard cold pressor test was evaluated in two hypertensive subjects; no significant UFA response was provoked by this stimulus.

c) UFA responses to nicotine: An increase in the incidence of coronary artery disease has been reported in cigarette smokers. UFA responses to cigarette smoking and nicotine infusions were studied as a factor.

Blood UFA levels increased significantly in 3 of 7 subjects smoking in an accustomed manner for 30 minutes. In the case of one of the reacting subjects a control run with nicotine-free cigarettes was performed revealing no UFA rise. These observations were inadequately controlled and suffer from the defect that nicotine exposure was variable and that provocation of an UFA response by emotional factors cannot be excluded.

In an attempt to standardize nicotine exposure 15 observations of UFA response to nicotine infusions were made in 7 subjects. It was hoped that it would be possible to find a dose of nicotine, free of symptoms in most patients, which would be capable of eliciting an UFA response. Two of 7 subjects receiving 2 mgm of nicotine bitartrate by infusion over 10 minutes had significant UFA rises without complaints of unusual symptoms; control saline infusions were negative. Two subjects received 4 mgm over a 10 minute period; both were symptomatic but showed no significant rise of UFA levels. The doses of nicotine used in these trials were relatively small compared to the amounts estimated to be absorbed by subjects who inhale cigarette smoke. The above observations while inconclusive, raise the possibility that nicotine can provoke increases in UFA levels.

d) Relation of UFA response to amine structure: Absence of an UFA response to dopamine has been found in four subjects who have shown pronounced responses to equi-pressor doses of the closely related amine, norepinephrine. A study of the amine structural configuration associated with an UFA response has been initiated and will involve use of dopamine, norepinephrine, epinephrine, tyramine, isoproterenol, and neosynephrine in doses producing equivalent vascular responses.

e) Relationship of cholesterol levels and UFA responses in man: Blood cholesterol elevations have been found in dogs receiving prolonged doses of epinephrine or norepinephrine at levels provoking marked UFA responses. These observations were extended in four human subjects who received eight hour infusions of norepinephrine at rates producing pronounced UFA responses. No significant elevation of cholesterol levels was found.

Proposed Course of Projects:

1. Study of the relationship of amine configuration to ability to provoke an UFA response will be continued.

2. The relationship of blood cholesterol elevations to UFA responses will be pursued in subjects receiving norepinephrine infusions after administration of small doses of hydrocortisone.

Part B included Yes No

Serial No. NHI-43
1. Gerontology Branch
2. Nutritional Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Age changes in the regenerative potential of various organs of the rat.

Principal Investigators: C. H. Barrows, Jr. (25% time)
D. A. Olewine (25% time)

Other Investigator: J. A. Falzone, Jr.

Cooperating Units: Baltimore City Hospitals

Man Years (1960):

Total:	1.177
Professional:	0.50
Other:	0.677

Project Description:

Objectives: The general purpose of this study is to determine whether (1) the amount of tissue produced during regeneration is different in senescent as compared to younger animals and (2) the tissue produced is biochemically different in the two age groups. Specific problems investigated during this period have been studies of kidney tissue following unilateral nephrectomy in rats of different ages.

Methods Employed: Unilateral nephrectomies were performed by the removal of one kidney by the dorsal route. The concentrations of various enzymes, DNA, RNA and protein nitrogen, were determined immediately after the removal of the kidney. Eight weeks following the operation, the animals were sacrificed, the remaining kidney removed and analyzed as described above.

Progress During Past Twelve Months: Unilateral nephrectomies were performed on twelve young (12 months) and twelve old (22 months) rats. The concentrations of alkaline phosphatase, succinoxidase, DNA, RNA and protein nitrogen per unit wet weight of these kidneys did not vary by more than 10% between two groups of animals. Eight weeks following the operation, the kidneys of the young animals

hypertrophied 44% whereas those of the old animals hypertrophied 33%. However, the concentrations of the various tissue components per unit wet weight in the hypertrophied kidney did not vary by more than 10% from those found in the normal kidney and no marked age differences were observed.

Direction of Current Research: Experiments will be continued on the biochemical changes in liver following partial hepatectomy. When adequate methods are obtained, the effect of age on the regeneration of liver tissue following single and repeated partial hepatectomies will be carried out.

Part B included: No

Serial No. NHI-44
1. Gerontology Branch
2. Human Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Measurement of the hemoglobin-haptoglobin complex clearance rate in man and animal.

Principal Investigators: J. Lowenstein (1/4 time; resigned 6/30/60)
D. Faulstick (1/4 time)
M. Yiengst (1/4 time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 1.625
Professional: 0.625
Other: 1.0

Project Description:

Objectives: The mechanism of clearance of hemoglobin-haptoglobin complex from the blood is being investigated in both man and animal. It is suspected that this is cleared by the reticulo-endothelial system.

Methods Employed: Several lines of investigation are being pursued: (1) Autogenous hemoglobin has been given to several human subjects and the clearance of the hemoglobin-haptoglobin complex followed. The clearance kinetics appear to follow a first order reaction and are similar to those noted in well described systems as colloidal carbon, chronic phosphate and heat denatured albumin which are cleared by the reticulo-endothelial system.

(2) Radioautographs of animal liver and spleen have been attempted using Fe^{59} hemoglobin. These have been unsuccessful to date.

Progress During Past Twelve Months: The clearance of hemoglobin-haptoglobin complex from the blood has been followed in 38 men. This was done in conjunction with renal clearance measurements of hemoglobin. The initial portion of the disappearance curve followed

an exponential course with a rate constant (K) averaging 0.00324 (S.D. 0.00107) and the product of the rate constant and dose (D) averaged 9.514 mg/kg body weight/min. (S.D. 0.245).

In this study the haptoglobin capacity was completely saturated and free hemoglobin was also present in the blood. It was felt that the true dose of hemoglobin-haptoglobin complex was possibly never known since the haptoglobin pool may be extravascular as well as vascular, allowing the extravascular element to enter the system and combine with the free hemoglobin. Twenty males have been given a single injection of hemoglobin to approximately saturate 85% of the haptoglobin binding capacity. The age range of subjects was from 54 to 81 years. Under these circumstances lower KD values were obtained with no significant age regression.

Direction of Current Research: (1) The effect of reticulo-endothelial stimulators (endotoxin) and blockers (thorotrast) on clearance rates are being checked in dogs.

(2) Cell separation techniques are being tried in an attempt to get a fairly pure Kupffer cell population. If this is successful, then the presence of hemoglobin-haptoglobin complex in these cells may be checked using rats and dogs.

(3) An attempt to vary the dose of complex presented to the clearance mechanism is also being tried to check the constant K x D over a wider range of D in humans.

Part B included: No

Serial No. NHI-45
1. Gerontology Branch
2. Nutritional Biochemistry
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Structure of the interphase nucleus.

Principal Investigator: J. A. Falzone, Jr. (1/4 time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 0.75
Professional: 0.25
Other: 0.50

Project Description:

Objectives: To investigate the structure of interphase chromosomes and the factors responsible for their condensation with mitosis.

Methods Employed: (1) Application of test reagents to isolated nuclei being observed by phase microscopy, (2) histochemistry and (3) differential centrifugation of nuclear fragments.

Progress During Past Twelve Months: Treatment of isolated nuclei from a variety of sources (rat liver, kidney, brain, onion stalks, Drosophila adults and larvae) with polycations (salmine, histone, poly-L-lysine) produces an effect resembling prophase. If this treatment is followed by polyanions (RNA, DNA, heparin, and several other substances) the nuclei become filled with small granules of rather uniform size and regular cubic or hexogonal packing. Often discrete double rows of slightly larger granules are apparent. Present morphological and histochemical information strongly suggests that these granules are complex coacervate droplets of applied polyelectrolytes adhering to the gyres of interphase chromosomes.

Attempts to chemically compare mitotic with interphase chromosomes (from regenerating and 'resting' rat liver, respectively) have not yet been successful. In the course of these experiments, however, we have obtained two morphologically distinct chromosome fractions by density gradient sedimentation.

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Direction of Current Research: (1) Completion of histochemical identification of nuclear granules, (2) Improvement of resolution of chromosome fractionation, (3) Chemical comparison of mitotic with interphase chromosomes, using rat liver or microorganisms dividing synchronously.

Part B included: No

Serial No. NHI-46
1. Gerontology Branch
2. Molecular Biology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Binding of cations to proteins.

Principal Investigator: Wilbur W. Everett (2/3 time)

Other Investigator: None

Cooperating Unit: Baltimore City Hospitals

Man Years (1960):
Total: 1.0
Professional: 0.666
Other: 0.333

Project Description:

Objectives: To determine the effect of metal ions on the conformation and net charge of proteins. Specifically, (1) to distinguish between the effect of different metal ions on the isomerization of bovine plasma albumin at low pH and (2) to elucidate the nature of the dimerization of bovine plasma albumin (BPA) brought about by copper under certain conditions.

Methods Employed: Solutions of the proteins in the absence of metal ions are compared with solutions containing metal ions in varying concentrations by ultracentrifugation, electrophoresis, and optical rotation.

Progress During Past Twelve Months: The effects of Cu^{++}, Co^{++}, and other ions on the isomerization equilibrium of BPA at pH 4 have been studied. Equilibrium constants for the binding of the ions are 3.60×10^4 for Cu^{++} and 6.84×10^3 for Co^{++} and the average numbers of binding sites are 2 to 3 for Co^{++} and 1 to 2 for Cu^{++}. The effect of the binding of both Co^{++} and Cu^{++} to BPA in the region of the isomerization equilibrium is to shift that equilibrium toward the low pH form of BPA. The effect of manganese on both the mobility of BPA in the acid region and on the isomerization equilibrium was found to be negligible.

In the course of determining the sedimentation constants of BPA under the conditions which were used to do the electrophoresis experiments a dimerization reaction was noted which has been found to be dependent on the presence of Cu^{++} ion. The dimerization was not observed in the presence of Co^{++}, Ni^{++}, Zn^{++}, Cd^{++}, or Cr^{+++}. The extent of dimerization has been found to be related to the copper to albumin ratio up to about 60-70% dimer, which is the maximum amount of dimer obtainable. This suggested that the group on the protein involved is the sulfhydryl of the cysteine residue. This was shown to be the case by the fact that blocking of the sulfhydryl with p-chloromecuribenzoate completely prevents the formation of dimer. The sedimentation constants of the monomer and dimer have been determined under a variety of conditions and some kinetic data have been obtained concerning the reaction.

Direction of Current Research: The dimerization reaction is being studied as to the effect of pH and ionic strength on the reaction. The binding data obtained by electrophoresis will be checked by a few equilibrium dialysis experiments.

Part B included: No

Serial No. NHI-47
1. Gerontology Branch
2. Comparative & Cellular Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Titles: Studies on relationships between development and aging: The normal histology and histochemistry of the various developmental stages of Drosophila from egg to fourteen day old adults.

Studies on relationships between development and aging: Nerve and muscle atrophy in the cockroach, Periplaneta americana.

Studies on relationships between development and aging: Humoral control of the accessory sex glands in the cockroach, Periplaneta americana.

Studies on relationship between development and aging: Studies on leg regeneration in the cockraoch, Periplaneta americana.

Studies on relationships between development and aging: Humoral control of ovary development in the cockroach, Periplaneta americana, and in the mosquito, Aedes aegypti.

The effects of aging on the developmental capacity of organ discs in the fruit-fly, Drosophila.

Principal Investigator: Dietrich Bodenstein

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: None
Professional: None
Other: None

PHS-NIH
Individual Project Report
1960

Project Description:

Progress During Past Twelve Months: All further research on these projects have been discontinued due to the resignation of Dr. Bodenstein.

Direction of Current Research: No future research planned.

Part B included: No

Serial No. NHI- 48
1. Gerontology Branch
2. Comparative & Cellular Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Studies on the relationship between proliferation and differentiation of skeletal muscle myoblasts in vitro.

Principal Investigator: I. R. Konigsberg (70% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 2.471
Professional: 0.700
Other: 1.771

Project Description:

Objectives: An evaluation of the postulate that growth (cell proliferation) and differentiation are mutually exclusive processes.

Methods Employed: Monolayer culturing of trypsinized cells, Leighton tube and roller drum culture (Human muscle), histology and histochemistry.

Progress During Past Twelve Months: Histochemical investigation of reductases in developing multinuclear cells has been pursued using the nitro-tetrazolium blue technique of Nachlas et al. Succinic dehydrogenase, DPN H-Cytochrome C reductase and TPN H-Cytochrome C reductase have been localized. All three enzymes increase dramatically concomittant with multinuclearity indicating an increase of mitochondrial number following cell fusion. In an effort to determine whether the multinuclear cell becomes dependent on oxidative pathways exclusively, cultures were grown in the presence of Antimycin A. The results indicate that although the mononucleated cell can maintain itself and proliferate in the presence of the blocking agent the multinuclear cells are selectively killed.

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Histochemical localization of reduced nucleotide reductases has also been useful in demonstrating cross-striations. These can be detected since the mitochondria are oriented along the A bands.

A comparison of human and embryonic muscle cells in culture indicates that cells originating from mature skeletal muscle tissue are also capable of neoformation of multinuclear muscle cells. After two-three weeks of culture long multinuclear ribbons form in the outgrowth area of the original explants. These resemble the early multinuclear cells of chick embryonic muscle having a highly refractile cytoplasm which upon proper fixation and staining can be demonstrated to contain many longitudinal fibrils. Removal of the original explants prior to multinuclear cell formation does not affect the process. This would indicate that the multinuclear cells do not arise simply by the "budding" of injured mature muscle cells.

Efforts are being made to isolate the various cell types found in mature muscle in an attempt to identify the source of the myogenic elements. Thus far attempts at disaggregation with a wide variety of enzymes have been unsuccessful.

Direction of Current Research: Histological and histochemical investigations of developing multinuclear cells are being continued to elucidate the factors controlling the formation of multinuclear cells and the role of multinuclearity in the further differentiation of the muscle cell.

Attempts to isolate mature muscle cells will be continued. Microsurgical techniques will be applied to supplement the enzyme disaggregation approach.

The feasibility of obtaining pure cultures of embryonic chick muscle cells will continue to be explored using metabolic inhibitors and immune sera prepared against chick fibroblasts.

Part B included: Yes

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Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Konigsberg, Irwin R., Norma McElvain, Martha Tootle, and Heinz Herrmann: The dissociability of deoxyribonucleic acid synthesis from the development of multinuclearity of muscle cells in culture. J. Biophys. Biochem. Cytol., 8: 333-343, Oct. 1960.

2. Konigsberg, Irwin R. On the relationship between development and aging. Newsletter (Geront. Soc.) 7:(3), 33-34, Sept. 1960.

Honors and Awards relating to this project:

Serial No. NHI-49
1. Gerontology Branch
2. Intermediary Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Studies on oxidative phosphorylation.

Principal Investigators: D. R. Sanadi (30% time)
A. Fluharty (100% time)
M. Fletcher (25% time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 2.716
Professional: 1.55
Others: 1.166

Project Description:

Objectives: To determine the role of dithiols in oxidative phosphorylation.

Methods Employed: The effect of specific dithiol inhibitors (e. g., arsenite and cadmium ions) on coupled phosphorylation and related partial reactions was studied.

Progress During Past Twelve Months: Arsenite uncoupled partially the phosphorylation coupled with the oxidation of succinate and β-(OH) butyrate in rat liver mitochondria. With the other common substrates, strong inhibition of oxidation was observed. However, in the presence of 2,3-dimercaptopropanol (BAL), arsenite was an effective uncoupling agent with all of the substrates. As is expected of an uncoupling agent, arsenite-BAL induced strong ATPase activity in mitochondria and inhibited the exchange of P^{32}-labeled inorganic phosphate with ATP. It is believed that BAL acts as a vehicle to carry the arsenite across an unknown barrier to the site of inhibition which may be another dithiol function on the enzyme complex. All of these effects of arsenite-BAL were reversed by the addition of an excess of BAL presumably because the arsenite is now displaced from the enzyme in favor of the attachment to BAL. BAL-arsenite complex produced swelling in mitochondria; however, uncoupling preceded the

swelling change. The swelling was unaffected but phosphorylation was restored by excess BAL. Similar results have been obtained with particles prepared by osmotic lysis of liver mitochondria.

Mitochondria from beef heart are similarly uncoupled by arsenite in combination with BAL.

A hypothesis implicating a dithiol at an early reaction in oxidative phosphorylation has been proposed.

Direction of Current Research: The effects of BAL-arsenite will be explored in fractionated oxidative phosphorylation systems in order to localize and isolate the sensitive dithiol grouping.

Part B included: Yes

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Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Fluharty, A. R., and D. R. Sanadi: Evidence for a vicinal dithiol in oxidative phosphorylation. Proc. nat. Acad. Sci., 46: (5), 608-616, May 1960.

Honors and Awards relating to this project:

Serial No. NHI-50
1. Gerontology Branch
2. Intermediary Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Studies on the oxidative decarboxylation of α-ketoacids.

Principal Investigators: D. R. Sanadi (40% time)
James Peters (50% time - E.O.D. 7/1/60)
Robert L. Searls (L.D. 6/30/60)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals and the American Heart Association

Man Years (1960):
Total: 2.15
Professional: 1.15
Other: 1.0

Project Description:

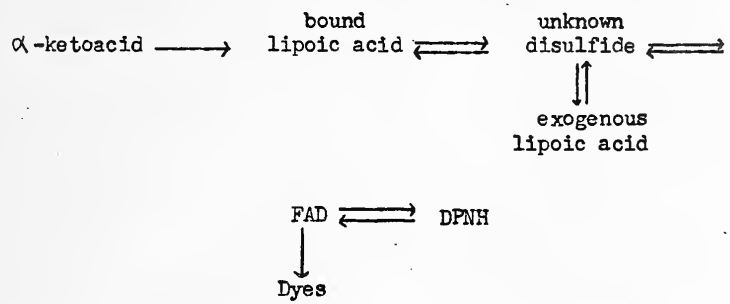
Objectives: Isolation of each of the components in the α-ketoglutaric dehydrogenase complex, study of the properties and eventual reconstruction of the over-all process with the individual units.

Methods Employed: Disintegration of the enzyme complex by relatively drastic procedures, isolation of the active components by classical protein fractionation methods and studies on the kinetics and mechanism of action of each constituent.

Progress During Past Twelve Months: The terminal step in the oxidative decarboxylation of α-ketoacids is the reduction of DPN by the reduced lipoic acid. This reaction is catalyzed by a flavoprotein which appears to be identical with Straub diaphorase.

The reduction of lipoic acid by DPNH, catalyzed by this enzyme, is strongly inhibited by arsenite and cadmium ions but the reduction of artificial electron acceptors is activated under the same conditions. The inhibited enzyme can be prepared by incubating the enzyme with its reducing substrate and the inhibitor. The results strongly suggest that the coenzyme has, besides flavinadeninedinucleotide (FAD), a

second oxidation-reduction group which is required for lipoic acid reduction but not for the reaction with dyes. The new "prosthetic" group is a disulfide which is reduced by DPNH or reduced lipoic acid to the dithiol. The sequence of electron transfer appears to be as shown below:



It was observed previously that the reduced flavoprotein had a new absorption peak at 530 mμ. This peak is now known to be due to an interaction between the enzyme dithiol group and FAD since addition of arsenite or cadmium ions results in its immediate disappearance. A model system which yields a similar peak has been discovered. Reduced lipoic acid and flavinmononucleotide (FMN), in the absence of any enzyme, react to form a complex with an absorption maximum at 530 mμ. On standing, the complex slowly breaks down to oxidized lipoic acid and reduced FMN. Electron spin resonance measurements showed no evidence for unpairing of electrons either in the model complex or in the enzyme reduced with DPNH.

Direction of Current Research: Attempts are being made to identify the disulfide present in the dihydrolipoyl dehydrogenase flavoprotein.

Part B included: Yes

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Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Searls, R. L., and D. R. Sanadi: Evidence for a vicinal dithiol in dihydrothioctyl dehydrogenase. Biochem. Biophys. Res. Comm., 2: (3), 189-192, March 1960.

2. Searls, R. L., and D. R. Sanadi: Oxidation of α-ketoglutarate by artificial electron acceptors. Biochem. Biophys. Res. Comm., 2: (3), 226-229, March 1960.

3. Searls, R. L., and D. R. Sanadi: α-Ketoglutaric dehydrogenase. VIII. Isolation and some properties of a flavoprotein component. J. biol. Chem., 235: (8), 2485-2491, Aug. 1960.

4. Searls, R. L., and D. R. Sanadi: On the mechanism of dihydrothioctyl dehydrogenase reaction. J. biol. Chem., 235: (8), PC 32-PC 33, Aug. 1960.

5. Sanadi, D. R., and M. J. Bennett: Synthesis of serine from glycine in mitochondrial fragments. Biochim. Biophys. Acta, 39: 367-369, April 1960.

Honors and Awards relating to this project:

Serial No. NHI- 51
1. Gerontology Branch
2. Human Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Age changes in renal physiology

Principal Investigators: J. Lowenstein (3/4 time - resigned 6/30/60)
M. J. Yiengst (3/4 time)
D. A. Faulstick (3/4 time)
H. M. Langs (Full time - E.O.D. 7/1/60)

Other Investigator: N. W. Shock

Cooperating Units: Baltimore City Hospitals

Man Years (1960):

Total:	5.916
Professional:	2.375
Other:	3.541

Project Description:

Objectives: To describe and elucidate the mechanisms of age changes in renal function.

Methods Employed: The measurement of glomerular permeability utilizing dextrans of various molecular weights has been performed. The methodology involving quantitation with a light scattering technic has been completed.

A method has been developed for estimation of glomerular clearance of hemoglobin based on the electrophoretic separation of free and haptoglobin bound hemoglobin. Standard methods are used in determining inulin and PAH clearances.

Progress During Past Twelve Months: Molecular weight distribution curves of dextran solutions have been successfully obtained using a light scattering technic. The methodology for the isolation and quantitation of dextran from blood and urine has been completed. Clinical dextran has been given to eight subjects and the distribution curves in the urine have been followed with time.

This pilot study of two groups, four young and four old men, was undertaken to check for any significant difference in glomerular permeability to various molecular weight fractions of dextran. The results of this study indicate that the only real difference between young and old men is the rate at which dextran is excreted. The maximum and mean molecular weight excreted is about the same in both groups. It is suspected that if a difference in permeability exists with aging, this technic will not demonstrate it.

Studies of glomerular clearance of hemoglobin relative to inulin have been carried out in 47 patients aged 20-88 years. The ratio C_{Hb}/C_{In} averaged 0.055 (S.D. 0.019) and showed no significant correlation with age. Tubular reabsorption of hemoglobin, calculated as the difference between filtered load and urinary excretion rate averaged 1.43 mg./min. (S.D. 0.96).

Studies on the renal function status of the volunteer longitudinal group, initiated in 1959, were continued. A small select group of Hospital and Infirmary subjects were tested by the same procedures - endogenous creatinine clearance and renal concentrating ability (urine osmolarity).

Serum albumin levels were determined in the volunteer groups over the past year. The data suggest an agewise decline in this value but no change was observed in serum globulins.

Direction of Current Research: Repeat measurements of renal function will be made after an interval of five years on subjects remaining in the Infirmary.

Estimates of creatinine clearance and concentrating ability with water deprivation will be continued in the group of volunteer subjects.

Part B included: Yes

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Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Gaffney, G. W., R. I. Gregerman, M. J. Yiengst, and N. W. Shock: Serum protein-bound iodine concentration in blood of euthyroid men aged 18 to 94 years. J. Geront., 15: 234-241, July 1960

Serial No. NHI-52
1. Gerontology Branch
2. Intermediary Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Effect on age on the turnover of mitochondria.

Principal Investigators: D. R. Sanadi (20% time)
Martin Fletcher (75% time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 1.448
Professional: 0.95
Others: 0.498

Project Description:

Objectives: To examine the possible dynamic turnover of mitochondria and mitochondrial components; to determine the effect of age of the animal on the half-lives of mitochondrial components.

Methods Employed: Groups of rats were administered S^{35}-methionine and C^{14}-acetate. They were sacrificed at weekly intervals and the liver mitochondria were isolated. The following components were fractionated from the mitochondria and counted for activity: soluble proteins, insoluble proteins, cytochrome c and lipids.

Progress During Past Twelve Months: The plot of the log of the specific activity against time was a straight line with each component. These lines were parallel and gave essentially the same regression coefficient suggesting that these four components turned over at the same rate. The data suggest that liver mitochondria are synthesized and broken down as a unit. There is no differential replacement of the constituents once the integrated structure is formed.

No significant difference was detected in the turnover rates of liver mitochondria between 12 month and 20-22 month old rats.

Direction of Current Research: Similar studies are planned with heart and brain mitochondria and with liver microsomes.

Part B included: No

Serial No. NHI-53
1. Gerontology Branch
2. Intermediary Metabolism Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Sulfhydryl groups in xanthine oxidase.

Principal Investigators: D. R. Sanadi (10% time)
James M. Peters (50% time - E.O.D. 7/1/60)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 0.35
Professional: 0.35
Other: None

Project Description:

Objectives: To determine whether dithiol groups are involved in xanthine oxidase.

Methods Employed: The effect of arsenite and cadmium ions on the enzyme activity was studied. The enzyme activity was determined spectrophotometrically as well as polarographically using the platinum microelectrode for measuring oxygen.

Progress During Past Twelve Months: Xanthine oxidase activity is inhibited strongly by arsenite (80% inhibition with 1×10^{-3} M arsenite). Hypoxanthine affords considerable protection if it is added to the enzyme before the inhibitor is added. However, the inhibition is non-competetive with the substrate. Arsenite inhibition is not reversed by 2-mercaptoethanol. 2, 3-dimercaptopropanol (BAL) inhibited the enzyme strongly. Cadmium chloride (1×10^{-3} M) did not inhibit xanthine oxidase activity. The results favor the conclusion that only monosulfhydryl groups are functional in xanthine oxidase.

Direction of Current Research: This project has been concluded.

Part B included: No

Serial No. NHI- 54
1. Gerontology Branch
2. Nutritional Biochemistry
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Pulmonary vascular elasticity.

Principal Investigators: W. R. Milnor (3/4 time - E.O.D. - 11/1/60)
J. W. Feeley (Full time - E.O.D. - 10/1/60)
J. S. Dodd (1/2 time - E.O.D. - 7/1/60)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals
Johns Hopkins Hospital and University
George Roveti, M. D., Department of Medicine,
Johns Hopkins University and Hospital
T. D. Lee, M. D., Fellow in Medicine,
Johns Hopkins University.

Man Years (1960):
Total: 0.6235
Professional: 0.4575
Other: 0.166

Project Description:

Objectives: To measure the distensibility of the normal pulmonary vascular bed, and its response to chemical and physical stimuli.

Methods Employed: The ratio of pulmonary blood volume to intravascular pressure is used as an index of over-all vascular distensibility. Pulmonary blood volume is measured by a modification of the Steward-Hamilton method, using successive injections of indocyanine green dye into pulmonary artery and left atrium to measure pulmonary mean transit time. Cardiac output is measured from the indicator-dilution curves, and pressures in pulmonary artery and left atrium by right and left cardiac catheterization.

In anesthetized, closed-chest dogs the effects of breathing room air, 100% oxygen, and 12% oxygen will be compared, and the response to epinephrine, nor-epinephrine, isoproterenol, and histamine will be tested.

In patients undergoing cardiac catheterization for diagnostic purposes the effect of negative pressure breathing (continuous negative airway

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1960

pressure of -5 to -10 cm. H_2O) is being measured, to study the relation between transmural pressure, volume, and vascular resistance.

Progress During Past Twelve Months: This project was initiated in the Gerontology Branch on 11/1/60. Laboratory facilities and equipment for the animal experiments have been procured and set in operation.

Direction of Current Research: Animal experiments on the effects of varying oxygen concentration as outlined above are under way. The human studies are in progress in the Cardiac Catheterization Laboratory of the Johns Hopkins Hospital.

Part B included: No

Serial No. NHI- 55
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: The relations between age and time estimation measures of accuracy, variability, and context effects.

Principal Investigator: David Arenberg (1/4 time - E.O.D. - 3/8/60)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 0.1875
Professional: 0.1875
Others: None

Project Description:

Objectives: Earlier studies have demonstrated age differences in time estimation. In addition, recent reports, including one from this laboratory, have implicated context effects as a source of age differences in perceptual performances (although in contradictory fashion), but direct evidence of such age related effects is not available. This study is designed to verify age differences reported in the literature, to determine the shape and extent of the relationship between accuracy and age (within the adult range), and to determine if, and in what way, context effects are related to age.

Methods Employed: Materials will be purchased and equipment built to permit programming three sets of eleven stimulus durations using log time as the dimension. One set will be symmetrical about one second, a second set will be asymmetric about one second with a prevalence of stimuli below one second, and a third set asymmetric about one second with a prevalence of stimuli above one second. A light will serve as the stimulus in the first study, but the equipment and method will be readily adaptable to other sensory modalities. The psychophysical method of single stimuli with two response categories will be used to derive three dependent measures: the period of a subjective second in a symmetric run (accuracy), intra-subject variability of this measure (discrimination), and shift of subjective second in the asymmetric runs (context effects).

Order effects will be handled by a counterbalancing design. Half of the subjects will be given early in the session a symmetric run immediately followed by an asymmetric run with a prevalence of stimuli above one second, and a symmetric run immediately followed by the other asymmetric run later in the session. The other half of the subjects will have the asymmetric runs in the other order. In other words, two orders will be used: AB interval AC and AC interval AB, where A is the symmetric run and B and C are the asymmetric. If feasible, these dependent measures will be related to time judgment measures shown to be age related in an ealier study.

Progress During Past Twelve Months: Stimulus periods have been selected, the design of the experiment has been determined, a subject group has been tentatively selected, and the materials necessary to build the equipment have been determined.

Direction of Current Research: Data collection will begin using only a visual stimulus. Later the project will be expanded to include other modalities. This will permit far greater generalization of experimental findings and also privode data for evaluating inter-modality relations.

Part B included: No

Serial No. NHI-56
1. Gerontology Branch
2. Comparative & Cellular Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Studies on energetic coupling systems.

Principal Investigators: D. D. Hendley (40% time)
B. L. Strehler (20% time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 1.10
Professional: 0.60
Other: 0.50

Project Description:

Objectives: To determine whether phosphate bond energy is utilized in the photoreduction of TPN.

Methods Employed: The rate of formation and utilization of ATP by spinach chloroplasts under various conditions was continuously measured using the very sensitive firefly assay system and new instrumentation developed in this laboratory. Measurements were also made of the exchange of P^{32}-labeled inorganic phosphate (P_i^{32}) with ATP in the presence of spinach chloroplasts.

Progress During Past Twelve Months: It has been found that there is a significant rate of P_i^{32} incorporation into ATP or other organic phosphates when spinach chloroplasts are illuminated in the presence of various oxidants, amounting to up to 25% of the rate of phosphorylation of ADP. However, the exchange rate does not appear to be greater with TPN than with other oxidants and there appears to be only a slightly greater rate of ATP hydrolysis in the light over that in the dark. While not conclusive, the data at least do not support the hypothesis that the photoreduction of TPN requires the utilization of high energy phosphate bond energy.

A marked inhibition of the exchange rate has been observed at higher levels of ATP (above 2×10^{-3} M) when TPN is the oxidant but not in the presence of phenazine methosulfate or ferricyanide.

The firefly assay system, as adapted for the continuous measurement of ATP levels in illuminated chloroplast suspensions appears to be a very useful new technique for studies in this field. While there are difficulties in the kinetic interpretation of the curves obtained, due to luminescence decay and the presence of myokinase, it has been possible to show that photophosphorylation may be initiated with virtually no lag when only AMP is added as phosphate acceptor suggesting that AMP itself may serve efficiently as substrate for photophosphorylation. A further observation of interest is a light catalyzed net utilization of ATP by chloroplast suspensions which can be observed on occasion under as yet unknown conditions. The exchange data would suggest, however, that such a light catalyzed hydrolysis may not occur to a significant extent concurrent with photophosphorylation.

Direction of Current Research: This project has been completed.

Part B included: No

Serial No. NHI-57
1. Gerontology Branch
2. Comparative and Cellular Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Studies of the effects of hibernation on cellular metabolism and senescence.

Principal Investigator: M. A. Brock (50% time - E.O.D. 10/3/60)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 0.125
Professional: 0.125
Other: None

Project Description:
Objectives: The current research is an extension and elaboration of previous work which showed that both erythrocyte senescence and metabolism were altered during deep hibernation of the golden hamster, Mesocricetus auratus. Aging of red cells was retarded; during hibernation their average length of life increased to approximately two times the control life span. Coincident with this change, the quantities of phosphorylated compounds found in the erythrocytes were significantly altered during hibernation: inorganic phosphorus increased with relation to control values; the phosphate in the fraction which contained primarily ATP decreased; there was an increase in the phosphate derived mainly from the 1-position of phosphorylated hexoses; and no changes occurred in the fractions which yielded phosphate primarily from diphosphoglycerate and the total phosphate. The identification of the specific reactions which are responsible for the altered metabolic activity and which are related to the change in the rate of senescence will be the direction of further research.

Methods Employed: The planning of the experimental methods to be used and the provision of housing for the experimental animals has been done during the last month.

Progress During Past Twelve Months: The last month and one-half has been spent in acquiring the materials, tools, animals, and methods necessary to carry out the above research.

Direction of Current Research: Ion exchange chromatographic techniques will be used to isolate glycolytic intermediates from erythrocytes of Mesocricetus auratus, utilizing warm room control animals, cold-exposed animals, hibernating animals and hibernators which have been aroused for six hours. These studies should lead to the identification of the specific enzymes which are responsible for the altered metabolic processes. The effect of body temperature on the enzyme activity will be described.

Part B included: No

Serial No. NHI-58
1. Gerontology Branch
2. Comparative & Cellular Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Studies on the occurrence, isolation and properties of lipofuscin age pigments from human cardiac muscle.

Principal Investigators: A. S. Mildvan (100% time - L.D. 9/1/60)
B. L. Strehler (20% time)
D. D. Hendley (60% time)
M. Reporter (Post-Doct. Res. Fellow)
(70% time E.O.D. 9/1/60)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals and Division of Research Grants and Fellowships, N.I.H.

Man Years (1960):

Total:	2.323
Professional:	1.725
Others:	0.598

Project Description:

Objectives: Determination of the chemical structure biochemical function and mechanism of formation of fluorescent particles that accumulate in the human myocardium with age.

Methods Employed: Quantitation of age pigment in myocardial slices, isolation, elementary analysis, lipid, protein, amino nitrogen analyses, and measurement of physical properties were made by procedures described in earlier reports.

Gas chromatography has been employed to effect a detailed separation of methylated components of age pigment lipids and of rancid cephalin.

Electrophoresis in a pH gradient has also been used to separate particulate fractions of heart homogenates and age pigment lipids.

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Individual Project Report
1960

Progress During Past Twelve Months: (1) By actual count under a microscope the proportion of yellow fluorescent particles to total particles was found to be surprisingly high; 50% even in crude homogenates of hearts from aged individuals. This could be increased to as much as 90% by the washing procedure described in an earlier report. Young hearts yeilded very small numbers of yellow fluorescent particles. More recently very pure age pigment preparations have been obtained by centrifugation after layering over sucrose and mechanical separation of layers in the resulting pellet.

(2) Measurements with an ocular micrometer of 40 age pigment particles in tissue sections indicate that the average particle has a major axis of 1.39 ± 0.06 (S.E.) micra and a minor axis of 0.97 ± 0.04 (S.E.) micra. By sedimentation in density gradients the density of age pigment would appear to be 1.18 ± 0.03, near that of mitochondria.

(3) Measurements of reduced vs. oxidized difference spectra indicate that age pigment particles isolated by the washing procedure contain little of the flavoproteins, cytochromes, or cytochrome c reductases present in mitochondria. Tests for some of the Krebs cycle oxidases also have yeilded negative results. On the other hand, cathepsin and acid phosphatase activities are pronounced and there are traces of acid deoxyribonuclease and ribonuclease. Thus the enzymatic behavior of age pigment appears to resemble that of lysosomes more than mitochondria.

(4) Fractionation of chloroform-methanol extracts of age pigment on silicic acid columns demonstrated a bluish fluorescence in a fraction characteristic of cholesterol esters while the yellow fluorescence characteristic of age pigment traveled with the cephalin fraction. The yellow fluorescent material shows an infra red spectrum very similar to that of old samples of animal cephalin which were also fluorescent. It was noted recently, however, that a more gradual elution of age pigment lipids from silicic acid columns reveals two distinct brownish components containing the bulk of the fluorescence. One of these contains so little ninhydrin reactive material that if it is a cephalin derivative the amino group has essentially disappeared (perhaps by reaction with aldehydic groups).

(5) Alkaline hydrolysis of age pigment lipids does not greatly alter the fluorescence. Following hydrolysis the fluorescent components

largely enter the aqueous phase when acidified and partitioned between hexane and water. Absorption and elution from charcoal appears to be a promising method for further purification of these water-soluble components.

(6) Gas chromatography of fluorescent column fractions subjected to methanolysis has revealed a preponderance of unsaturated over-saturated fatty acid esters. The fluorescent components themselves, however, may be largely retained by the column packings thus far employed judging by the behavior of various rancid cephalin fractions from which very little fluorescent material emerges from the column.

(7) Electrophoresis in a pH gradient shows promise in separation of lipid mixtures in non-polar solutions or in aqueous dispersions. Heart particulates have also yielded discrete fractions in this system although as yet there has been little concentration of age pigment particles.

Direction of Current Research: (1) Further efforts will be made to identify fluorescent components of age pigment lipids degraded by chemical and enzymatic procedures.

(2) Further comparison of enzymatic activities of age pigment particles and those of mitochondria and lysosomes is planned.

(3) Attempts will be made to induce yellow fluorescence in age pigment particles and lipid components from young hearts by oxidation and to compare properties of this material with that isolated from old hearts.

(4) A chemical comparison of age pigments and of artificially induced pigment bodies is projected.

Part B included: No

Serial No. NHI- 59
1. Gerontology Branch
2. Comparative & Cellular Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Studies on changes in localization and/or concentration of molecular populations during development, maturation and aging.

Principal Investigators: B. L. Strehler (20% time)
I. R. Konigsberg (30% time)
M. Reporter (Post Doct. Res. Fellow) (30% time- E.O.D.-9/1/60)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals and NIH Division of Research Grants and Fellowships

Man Years (1960):

Total:	1.342
Professional:	0.575
Other:	0.767

Project Description:

Objectives: General. These studies were undertaken in order to measure small amounts of materials in individual cells, their localization and concentration, etc. The measurement of the absorption spectra of single age pigment particles in situ (sections) is one specific objective. A study of ploidy variation and other possible cytological effects of DNA change with age are projected.

Methods Employed: Spectrophotometry, histochemistry and time lapse cinematography.

Progress During Past Twelve Months: The recording spectrophotometer has been modified to permit continuous scanning of the spectrum. This modification has made it feasible to run complete absorption curves on each test object. Comparisons of absorption curves with base line traces permit corrections to be made of scattering artifacts. Applying such corrections plus a correction for self-absorption (two-wave length method of Ornstein and Patau) reduces the standard error

of replicate readings (10-12 readings per test object) to between 1-3% of the mean.

Measurements of ploidy variability in developing chick muscle cultures have been undertaken to compare the distribution in multinuclear cells with that in mononuclear cells.

Preliminary results indicate a unimodal distribution of DNA values for the muscle cell nuclei which corresponds to the lower peak (diploid) of the bimodal distribution found in the mononuclear cells. If this can be confirmed it would support the evidence that muscle is a true syncytium.

Direction of Current Research: Ploidy measurements on cultured cells will be continued to determine whether the nuclei of the multinuclear muscle cells are, in fact, non-proliferative.

Measurements of DNA per cell will be extended to comparisons of cells of old and young organisms to test the premise that the frequency of mitotic "accidents" increases with age. (Frequency of mongolism with age seems to indicate increased frequency of nondisjunction which may have a mitotic parallel.)

Time lapse studies of the development of birefringence will be continued to elucidate the spatial pattern of myofibril growth.

Part B included: No

Serial No. NHL-60
1. Gerontology Branch
2. Comparative & Cellular Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Studies on mathematical-physical expressions of population mortality.

Principal Investigators: B. L. Strehler (No time)
A. S. Mildvan (No time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: None
Professional: None
Other: None

Project Description:

Objectives: To induce and deduce appropriate equations and assumptions to account for the shape of mortality curves.

Methods Employed: A theoretical and mathematical approach is used.

Progress During Past Twelve Months: This work was completed.

Direction of Current Research: Since this phase of the work is completed, the project is terminated with the current report. Several publications have resulted or are in press.

Part B included: Yes

PHS-NIH
Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Mildvan, A. S., and B. L. Strehler: A critique of theories of mortality. In: B. L. Strehler (Editor), The Biology of Aging. American Institute of Biological Sciences, Washington, 1960, Publ. #6, pp. 216-235.

2. Strehler, B. L.: Fluctuating energy demands as determinants of the death process. In: B. L. Strehler (Editor), The Biology of Aging. American Institute of Biological Sciences, Washington, 1960, Publ. #6, pp. 309-314.

3. Strehler, B. L., and A. S. Mildvan: General theory of mortality and aging. Science, 132: 14-21, July 1960.

4. Strehler, B. L.: Dynamic theories of aging. In: N. W. Shock (Editor), Aging: Some Social and Biological Aspects. American Association for the Advancement of Science, Washington, 1960, Publ. #65, pp. 273-303.

5. Strehler, B. L. (Editor): The Biology of Aging. American Institute of Biological Sciences, Washington, 1960, Publ. No. 6, vii, 364 pp.

Serial No. NHI- 61
1. Gerontology Branch
2. Comparative & Cellular Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Studies on the effects of temperature and other environmental factors on the aging process.

Principal Investigator: B. L. Strehler (20% time)

Other Investigator: Owen Sear (50% time - 3 month student volunteer)

Cooperating Units: Baltimore City Hospitals and Department of Medicine, Johns Hopkins University Medical School

Man Years (1960)

Total:	1.47
Professional:	0.325
Other:	1.145

Project Description:

Objectives: To study quantitatively the effects of temperature and other environmental factors on the aging process in invertebrates.

Methods Employed: The studies consist of measurements of the actuarial behavior of Drosophila melanogaster reared under controlled environmental conditions and subjected to quantitated physical or chemical stresses. During the past year the effects of the following factors have been investigated: low O_2, high O_2, bacteria free environments, radiation, α-tocopherol and heavy water.

Progress During Past Twelve Months: Radiation (50,000 r) increases longevity by 50% in non-sterile environments, but decreases it about 20% under sterile conditions. Flies free of microorganisms live somewhat longer than non-sterile animals. A 100% O_2 atmosphere increases the rate of aging about 50% whereas a 2% and 1% O_2 atmosphere decrease the rate of the process by about 35% as measured by average longevity and rate of increase of mortality. Heavy water in 20 and 40% concentrations decreases longevity about 50%.

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1960

Direction of Current Research: It has been found that a great many structures in the imago of Drosophila are brilliantly fluorescent. A study of the anatomical changes occurring under the influence of various environmental factors and of time is being initiated. In addition, other variables, such as antioxidants and dietary restriction, will be explored for their effect on longevity.

Part B included: No

Serial No. NHI-62
1. Gerontology Branch
2. Comparative & Cellular Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Studies on the comparative physiology of senescence: Investigations on the regression of Campanularia, Pennaria, Bougainvillia and other coelenterates.

Principal Investigator: B. L. Strehler (20% time)

Other Investigators: Owen Sear (50% time - 3 month student volunteer)
Mary Ann Brock (50% time - E.O.D. - 10/3/60)

Cooperating Units: Baltimore City Hospitals and Department of Medicine, Johns Hopkins University Medical School

Man Years (1960):
Total: 1.20
Professional: 0.45
Other: 0.75

Project Description:

Objectives: The phylum coelenterata contains many closely related species with widely varying longevities--from a few days to a century or more. An understanding of the behavioral, anatomical, biochemical and histochemical precursors and attendants to the death process in these animals is the general objective of this research.

Methods Employed: Manipulation of the environment (radiation, temperature) microsurgery, transplantation, histochemistry and direct observation through time lapse photomicrography.

Progress During Past Twelve Months: Extensive time lapse records of the development, function, senescence and death of a variety of species of coelenterates, including particularly the calyptoblastic colonial hydroid, Campanularia flexuosa, Clytia, Bougainvillia, Pennaria, Astrangia danae, Hydractinia, Sagartia lucia and Metridium senile have been made. Stolonic fusion, hydranth regression, digestion, respiratory movements, growth, cleavage and other functions have been photographed.

The regular senescence and death of Campanularia begins with a grad-

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1960

ual contraction of the tentacles and is followed shortly by a lysis and contraction of the hydranth proper, with the return of its contents to the colony.

50,000 to 200,000 r of X-rays results in a doubling of life expectancy from about 2.7 days to 5.8 days. The effect of anaerobicity, temperature and other environmental variables has been explored.

Histological and histochemical examination has shown a gradual thickening of the mesogleal layer in many older individuals.

Direction of Current Research: An intensive examination of histochemical properties and ultrastructural details is currently being undertaken in collaboration with Dr. Brandes of the Pathology Department of the Baltimore City Hospitals.

Part B included: No

Serial No. NHI-63
1. Gerontology Branch
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: The role of sulphydryl groups in enzyme action.

Principal Investigator: J. J. Blum (75% time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 1.75
Professional: 0.75
Other: 1.00

Project Description:

Progress During Past Twelve Months: Previous results established the general features of the reaction of Zn++, Cu++, Cd++, and PCMB (parachloromercuribenzoate) with myosin A. The similarities and differences between these reagents with respect to their effects on ATPase and ITPase activity have been investigated as a function of pH, temperature, and time of pretreatment. The participation of at least two -SH groups in the enzymatic process seems certain. It appears that one -SH group, which reacts very rapidly with PCMB, Cu++, Cd++, or Zn++, is responsible for the activation of ATPase and inhibition of ITPase activity. A second group, which reacts more slowly with these reagents, inhibits the activity of myosin with respect to either type of substrate. As a direct outgrowth of this work on the role of -SH groups in the contractile proteins, we have begun an examination of the role of -SH groups on other enzymes - lactic dehydrogenase (LDH) in particular - where it is known that changing part of the coenzyme (DPN) from an adenine group to an inosine group affects the enzymatic activity. We have found that there is an -SH group on the active site of LDH. The access of PCMB to this -SH group is, however, not controlled by the adenine or inosine part of the DPN, but by the phosphate group closest to the nicotinamide end of the DPN molecule. We have also found that some of the effects of Cu++, Cd++, Zn++, Co++, and Ni++ on LDH are similar to some of the effects of these cations on myosin.

Direction of Current Research: We are presently examining the effects of trypsin and of heat on the activity of LDH to see whether there is any evidence that the adenine part of the DPN coenzyme molecule causes a conformation change in LDH and to see whether the -SH group on the active site contributes to the stability of the enzyme. These factors have already been examined in the case of myosin A, and there we are in a position now to attempt to assign roles in the contractile process to the -SH groups that are involved in the enzymatic process.

Part B included: Yes

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Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Blum, J. J.: Interaction between myosin and its substrates. Arch. Biochem. Biophys., 87: 104-119, March 1960.

2. Blum, J. J.: Concentration profiles in and around capillaries. Amer. J. Physiol., 198: 991-998, May 1960.

Honors and Awards relating to this project:

Serial No. NHI-64
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Reaction time and electroencephalographic alpha frequency as functions of aging.

Principal Investigator: W. W. Surwillo (1/2 time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 0.6975
Professional: 0.50
Other: 0.1975

Project Description:

Objectives: To investigate age decrement in simple reaction time with reference to age changes in the alpha frequency of the electroencephalogram. We hypothesize that the reaction-time differences between age groups can be accounted for, in part, by differences in the alpha frequencies observed between the two groups. We also hypothesize and hope to show that the frequently reported variability in simple reaction time, observed in the same individual over a group of trials, is a function of the alpha frequency present in the interval between stimulus and response.

Methods Employed: A simple reaction time procedure, in which the subject responds to the appearance of an auditory stimulus (250 cycle tone, well above threshold) by pressing a button with the thumb, is used. Alpha waves in the interval of time between stimulus and response are counted and the average period of the alpha rhythm in this interval is determined for each stimulus-response pair. Reaction time and alpha period measures are obtained from subjects over the entire age span.

Data from the group, i.e., mean reaction times, mean alpha periods, and age, will be subjected to a multiple regression analysis. A multiple correlation coefficient will be computed in which reaction time is treated as the dependent variable and alpha period and age

as independent variables. This analysis will reveal what proportion of the variance in mean reaction times from the whole group can be accounted for by alpha period, and what proportion can be accounted for by age.

Data from individual subjects are analyzed in conjunction with recordings of spinal reflex, motor time, and instantaneous heart rate (see Project Report Serial No. NHI-66). These variables are employed collectively as measures of central excitatory state and are used in the analysis to reduce the reaction time variance. Correlations between reaction time and alpha period--with spinal reflex, motor time, and instantaneous heart rate partialed out--are computed for each subject.

Progress During Past Twelve Months: Data from group. Analysis has not been started since data from a sufficient number of subjects have not yet been collected and tabulated. Analysis of data from the pilot investigation revealed a correlation of 0.81 ($P < 0.01$) between mean reaction time and mean alpha period in a group of 13 subjects.

Data from individual subjects. Correlations (direct, not partial) between reaction time and alpha period in individual subjects, for a sample of 10 of the total number of subjects on which data are available, were found to range from 0.03 to 0.65. A regression analysis was then run on the data from one subject which had yielded the correlation of 0.03. The partial correlation between reaction time and alpha period--with amplitude of spinal reflex, heart rate, and motor time (time which elapses between the appearance of a muscle action potential and the mechanical response of the muscle) held constant--proved to be 0.29, a substantial increase.

Since partial correlations are required for every individual in the sample, the statistical processing was put in the hands of the Computation and Data Processing Branch of the Division of Research Services. To date the analysis on one case has been delivered.

Direction of Current Research: Pending outcome of the above analyses, plans are underway to study the relation between alpha period and the rate at which stimulus information is processed in a choice reaction time task.

Part B included: Yes

PHS-NIH
Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Suci, G. J., M. D. Davidoff, and W. W. Surwillo: Reaction time as a function of stimulus information and age. J. Exp. Psychol., 60: 242-244, 1960.

Honors and Awards relating to this project:

Serial No. NHI-65
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Verbal learning and age.

Principal Investigator: David Arenberg (1/2 time - E.O.D. 3/8/60)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 0.436
Professional: 0.375
Other: 0.061

Project Description:

Objectives: The broad objectives involve determining the factors which affect verbal learning performance at various adult age levels. In terms of applied research this can be re-stated as an attempt to discover the conditions for optimal verbal learning performance of the elderly. The immediate objective is to evaluate the effects of imposed pacing and self-pacing. Two primary hypotheses will be tested: (1) learning performance, over the range of imposed pacing used in these studies, is directly related to slowing of pace with performance under self-pacing even better than the slowest imposed pace; (2) older subjects benefit even more than do younger subjects with slowing of the imposed pace and most of all under conditions of self-pacing; i.e., between age and pacing there is an interaction effect in which the superiority of the performance of the young compared with the old subjects decreases with slowing of pace and is least under self-pacing.

Methods Employed: Serial learning and paired associate learning studies are under way. In each study analyses will be made for two dependent measures--number of trials to criterion and number of errors. The independent variables are age and pace. The pace dimension consists of two, three, and four seconds and self-pacing. The age dimension is divided into young and old (with a middle category if the sample size permits). Subjects include those from the longitudinal program and paid subjects. If the same procedures can be

used for both high and low groups as measured by WAIS Vocabulary score, this dimension will be dichotomized and included as an independent variable with two levels. If not, procedures will be modified for the low group constituting a separate study (with the same design).

The learning criterion for both studies is one perfect trial. In addition to original learning, the paired associate study includes learning a second set of response words to the original stimuli followed by re-learning of the original set. This permits evaluation of proactive and retroactive effects.

Progress During Past Twelve Months: Initial conceptualizations of this project were made in April. Since then, new equipment was purchased and put into working order, two modes of verbal learning were selected, stimulus material was selected and prepared, pacing conditions were determined, potential subject groups were specified, special instructions were written, practice subjects were run, and data have been collected for the first experimental subjects.

Direction of Current Research: For the immediate future, data will be collected under the conditions specified above. Later, studies in which other variables (such as kinds of stimulus material, meaningfulness, redundancy, length of list, distribution of practice, and learning criteria) are evaluated may be added.

Part B included: No

Serial No. NHI-66
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: A study of central nervous system factors in aging deficit.

Principal Investigator: W. W. Surwillo (1/2 time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 0.561
Professional: 0.5
Other: 0.061

Project Description:

Objectives: To determine the relation between motor deficit in aging and the activity level of the Brain Stem Reticular System (BSRS). It is hypothesized that the activity level of the BSRS is reflected at the periphery in the amplitude of the spinal reflex. The present aim of this project is to relate amplitude changes in the spinal reflex to changes in speed of response and thence to the decrement in response speed which is associated with aging.

Methods Employed: As pointed out in last year's annual report this project has, in terms of the collection of data, been combined with the project entitled "Reaction Time and EEG Alpha Frequency as Functions of Aging." Amplitude of the spinal reflex is recorded at one-second intervals while a subject is occupied with the task of responding to the appearance of an auditory stimulus by pressing a button. Since the testing session is spread over a period of time, and no forewarning is given to announce the presentation of the stimulus, the subject's responses are studied over a wide range of activity status from near-sleep to high-level attention. Instantaneous heart rate and motor time are recorded simultaneously with the spinal reflex recordings as they have proven to be significantly correlated with the latter measure. Motor time is the time which elapses between the appearance of a muscle action potential, and the mechanical response of the muscle.

Multiple correlation coefficients in which reaction time is treated as the dependent variable and amplitude of spinal reflex, heart rate, and motor time as independent variables are computed for each subject; the contribution of spinal reflex to the total reaction time variance is determined. When a sufficient number of cases becomes available, the group data will be analyzed to determine the contribution of spinal reflex to reaction time differences with age.

Progress During Past Twelve Months: Considerable time has been expended in measurement of recordings and tabulation of data. Statistical processing of the data is being handled by the Computation and Data Processing Branch of the Division of Research Services. To date a single case has been processed and delivered. From this and from other preliminary analyses it seems clear that the variables studied (amplitude of spinal reflex, heart rate, and motor time) can account for a significant portion (1/3) of the reaction time variance. The contribution of amplitude of spinal reflex to this variance appears to be significant but any conclusions must await analysis of more cases.

Direction of Current Research: The coming period will be spent in measurement and analysis of available data and in the collection of data from more cases.

Part B included: No

Serial No. NHI-67
1. Gerontology Branch
2. Human Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Care of patients, housed on Ward B-2 (60 beds), who participate in studies performed in this unit. In addition, all males acceptable for admission to Baltimore City Hospitals Infirmary receive medical evaluation and assignment to appropriate facilities in the Baltimore City Hospitals for treatment.

Principal Investigators: Albert Mildvan (1/1/60-6/30/60)
Fred F. Johnston, Jr. (7/1/60-12/31/60)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals staff and Clinical Laboratories

Man Years (1960):
Total: 1.0
Professional: 1.0
Other: None

Project Description:
Progress During Past Twelve Months: Assigned January 1, 1960 through December 31, 1960.

Direction of Current Research: Stated above.

Part B included: No

Serial No. NHI-68
1. Gerontology Branch
2. Nutritional Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Age studies of cell particulates and fractions.

Principal Investigator: J. A. Falzone, Jr. (1/2 time)

Other Investigators: C. H. Barrows, Jr.
N. W. Shock

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: This project is designed to measure age changes in the morphology, chemistry, and function of separable protoplasmic particles. The working hypothesis of these studies is that overall cell function may be well maintained until the moment of cell death by virtue of compensatory adjustments in the activities of its parts.

Methods Employed: Animals used will generally be the same as for Project No. 65; i. e., young adult and old rats aged 12 and 24 months, respectively. The basic technique is that of differential centrifugation of tissue homogenates at 0°-4°C. using an ordinary or ultracentrifuge depending upon particle size. Other techniques required by special problems are indicated below.

Progress During Past Twelve Months: Succinoxidase per unit washed mitochondrion was not different with age in either liver or kidney if mitochondrial protein nitrogen or turbidity of washed suspensions were used as the indices of mitochondrial number. The total evidence thus suggests a senescent loss of whole mitochondria from kidney rather than gradual loss of enzyme per mitochondrion. The validity of this conclusion can only be tested by adequate mitochondrial counts. Preliminary work suggests that mitochondria can be collected on the surface of millipore filters for more simple and accurate counting than is possible in counting chambers.

Such experiments in the field of senescence would be of greater interest if applied to post-mitotic cells such as neurones. However, presently available mitochondrial preparations from brain are heavily contaminated with myelin particles and probable glial material. Incubation of minced cerebellum with papain and versene yields a suspension containing free Purkinje cells, which can probably be isolated for metabolic studies and mitochondrial preparation.

Direction of Current Research: (1) Perfection of mitochondrial counting technique.

(2) Isolation of whole cells (Purkinje, liver) for metabolic studies and mitochondrial preparation.

Part B included: Yes

PHS-NIH
Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Barrows, C. H., Jr., J. A. Falzone, Jr., and N. W. Shock: Age differences in the succinoxidase activity of homogenates and mitochondria from the livers and kidneys of rats. J. Geront., 15: (2), 130-133, April 1960.

Honors and Awards relating to this project:

Serial No. NHI-69
1. Gerontology Branch
2. Nutritional Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Age changes in the intact rat.

Principal Investigator: D. A. Olewine (3/4 time)

Other Investigator: C. H. Barrows, Jr.

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 2.2916
Professional: 0.75
Other: 1.5416

Project Description:

Objectives: The objectives of this program are to study physiological changes in the senescent rat. At present two specific areas are under investigation:

(1) Experiments are being conducted on the activity of ad libitum fed rats and dietary-restricted rats. Since dietary restriction prolongs life span a comparison of activity in these animals with the activity of older and younger ad libitum fed rats is of great interest.

(2) Techniques are being developed for measuring renal function in the senescent rat. Because rat tissues are readily available for examination for disease, cell loss, and enzymatic change, a study of this kind has a definite advantage in that these data may provide direct information as to the cause of any functional change.

Methods Employed:

(1) Activity measurements

(a) Animals--activity was measured in three groups of male rats:

(1) 9 six month old ad libitum fed rats.

(2) 8 six month old rats which from the age of one month were fed 1/2 the food consumed by the ad libitum group.

(3) 6 two month old ad libitum fed rats weighing the same as the restricted group.

(b) Equipment

(1) Suspension type cage - This records all activity which causes the cage to move in a vertical plane such as locomotion, standing on hind legs, grooming, scratching, etc. Attempts to compensate for differences in body weight between the ad libitum and restricted rats (384 gm. and 200 gm. respectively) were carried out by the addition of lead ballast to the cages.

(2) Wheel cage - This type records volitional activity in the wheel only. All other movements such as occur within the housing cage attached to the wheel are not recorded.

(2) Renal clearance - The method thus far developed to measure renal clearances in the unanesthetized rat is as follows:

The day before the experiment plastic tubes are placed in the right jugular veins and left carotid artery. On the day of the experiment the animal is lightly anesthetized with ether and the bladder catheterized. Blood levels are maintained by a constant intravenous infusion of PAH and inulin. An arterial blood sample is obtained at the midpoint of each 10 minute clearance period and the period terminated by washing out the bladder. At least three consecutive clearance periods are obtained in each rat.

Progress During Past Twelve Months: (1) Activity measurements - In 9 ad libitum fed rats and 8 restricted rats the mean activity for 24 hours was 1103 units and 283 units respectively. It is unlikely that the difference in body weight between these two groups could account for these results, as the mean activity for 24 hours in 6 younger ad libitum rats of the same body weight as the restricted was 621 units. Although these data may support the concept that the activity of the restricted rat is more like that of a chronologically younger animal, other data are contrary to this. The day/night ratio of the rates of activity was the same in both groups of ad libitum rats (0.40) but markedly higher in the restricted rat (1.25).

Since decreases in activity measured in a similar type cage have been reported in the literature for starved rats, these data suggest that the restricted animal may resemble the hungry rat rather than the chronologically younger one. Data obtained in the wheel cage further support this conclusion.

In 9 ad libitum rats and 8 restricted rats of the same age the activity for 24 hours was 106 revolutions and 509 revolutions respectively. This difference resulted primarily from an increased activity rate during the day in the restricted group.

The hunger drive is known to increase wheel running and observations taken periodically throughout the day indicated that rate of activity increased in the restricted rats as feeding time approached. By changing the feeding time of the restricted rats from 4 P.M. to 7 A.M., the day/night ratio of activity rates was reduced from 3.64 to 1.34.

Thus these data indicate the marked influence of the hunger drive on the activity patterns of the restricted animals.

(2) Renal clearance - Using the method described it is now possible to measure renal clearances in undisturbed rats without anesthesia and under conditions in which inulin and PAH plasma levels remain constant for 30 minutes. By this method P_{PAH} has been maintained at 3 mg% for three clearance periods and then raised to 10mg% for an additional 30 minutes. Since P_{inulin} remained at 20 mg% throughout this time, it has been possible to calculate glomerular filtration rate (GFR), renal plasma flow (RPF), and tubular maximum (T_m) in the same individual.

The results of the first experiment are:

(a) GFR - 0.58 ml/min/100 gm.
(b) RPF - 2.09 ml/min/100 gm.
(c) T_{mPAH} - 0.10 mg/min/100 gm.

(3) Animal colony - In order to increase the number of senescent rats available for research a breeding program was recently started with rats of the Wistar strain. More rigid cleaning procedures which include strict isolation of the Wistar rats also have been put into practice. It is hoped that these procedures will reduce the incidence of respiratory infections in the colony.

Direction of Current Research: (1) The activity studies will be expanded to include ad libitum fed rats of different ages as well as rats whose food intake has been restricted by various means.

(2) Efforts will continue to be made toward developing a more reliable method for determining renal clearance in the rat.

Part B included: No

Serial No. NHI-70
1. Gerontology Branch
2. Nutritional Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Age as a factor in experimentally-induced atherosclerosis in the rat.

Principal Investigator: R. B. McGandy (2/3 time - E.C.D. 6/20/60)

Other Investigator: C. H. Barrows, Jr.

Cooperating Units: Baltimore City Hospitals and Maryland State Health Department

Man Years (1960):
Total: 0.386
Professional: 0.386
Other: None

Project Description:

Objectives: To investigate the possible role of age in experimentally-induced hypercholesterolemia and atherosclerosis in the rat, and to investigate the causes underlying age-related differences in susceptibility.

Methods Employed: High fat, cholesterol-cholic acid diets will be fed to groups of rats of different ages. The rate and extent of change in plasma lipid levels and in vascular sudanophilia will be compared among the age groups. Certain tissue and plasma lipid and enzyme levels will be determined in order to identify changes associated with hypercholesterolemia, and vascular sudanophilia.

Progress During Past Twelve Months: Although the extent of vascular sudanophilia has not yet been quantitated, a preliminary experiment has produced a five-fold increase in plasma cholesterol in 1 year old rats. Since it is known that the liver plays a role in the regulation of plasma lipids, some observations were made on liver tissue in this study. Mean liver weight to body weight ratios were increased to 3.33 in the high fat, cholesterol-cholic acid group (hypercholesterolemic) as compared to 2.66 in the high fat alone or 2.70 in the chow control groups. Succinoxidase activity in liver was reduced 40% on

a wet weight basis in the hypercholesterolemic group. That this reduction of succinoxidase still exists on a "per cell" level was substantiated by measurement of liver tissue D.N.A. Admixture experiments showed that the decreased succinoxidase "per cell" is apparently not due to inhibition of this enzyme.

In order to examine changes in the metabolism of the artery wall associated with lipid accumulation, aortic tissue endogenous oxygen uptake has been measured. This technique has been adapted to the use of a 50 mg. segment of thoracic aorta, thus allowing duplicate determinations on the same rat. Values so far obtained for endogenous oxygen uptake in normal rat aorta--with and without succinate stimulation--are comparable to reports in the literature based on experiments in which the entire thoracic aorta has been used.

Direction of Current Research: Micro-methods for measuring cholesterol, phospholipid, and triglyceride in rat tail blood, and for estimating free and esterified cholesterol in liver are being developed.

Experiments to determine which dietary components lead to the decreased succinoxidase levels in liver are in progress. Also, the effects of exogenous thyroxine and magnesium on these findings are being investigated.

Part B included: No

Serial No. NHI-71
1. Gerontology Branch
2. Psychology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Memory functions as related to age.

Principal Investigator: Melvin Davidoff (full time - resigned 9/2/60)

Other Investigator: David Arenberg (1/4 time - E.O.D. 3/8/60)

Cooperating Units: Baltimore City Hospitals
George Suci, Cornell University, Ithaca, N. Y.

Man Years (1960):

Total:	1.446
Professional:	0.8535
Other:	0.5925

Project Description:

Objectives: To study memory for different types of material and sense modalities as a function of aging. Two hypotheses are presently under investigation: (1) Aged subjects' short span memories are more susceptible to interference than younger subjects and (2) the retention of verbal sequences is a function of sequence length, redundancy in the sequence and age.

Methods Employed: (1) The amount of interference as measured by "interference" on judgments of sizes of squares was to be observed for subjects of different ages. Subjects learned to judge the relative size of a set of five cardboard squares to a certain criterion. The experimental group was then asked to judge the size of another set of five squares, each of which was larger than any of the squares in the first set. They were then asked again to judge the relative size of the first set of squares. The control group was not exposed to the "interfering" set. Instead, there was merely a conversational recess between the first and second presentation of the original set of cards, this recess being equivalent in time to the average time taken for second set presentation in the experimental group. The variable measured

in this study is number of trials to the learning criterion. This study, involving 96 subjects, gave unequivocally positive results. The subjects studied, it should be noted here, were all people with less than a complete high school education.

(2) Word lists, ten, fifteen, twenty and twenty-five words in length, and of varying redundancy (such that each word occurs independently of the last in the list, two words are dependent but independent of the next pair, and so on through seven word dependency) were constructed. Each list was read to the subject at the rate of one word per second. The subject was asked to repeat as many words as he could remember. Scoring is in terms of the number of words recalled correctly. Analysis is in terms of the interactions and primary effects of the variables of age, length and redundancy. Appropriate controls re possible effect of order of presentation of the word lists are incorporated in the study.

Progress During Past Twelve Months: Data have been collected for two new size judgment experiments in which it was necessary to increase the number of cards from five to six because of the higher caliber of subjects (mostly professional and executive level from the longitudinal sample). The difference between these two experiments is that in the first, twenty "interfering" stimulus presentations were made, whereas in the second, thirty were made. In the first, the sample size was eighty-nine (sixty-one young and twenty-eight old); in the second, a total of fifty-five subjects were run (thirty-three young and twenty-two old). As previously reported for a group with lower educational attainment, the old subjects showed somewhat greater interference, but the difference between old and young subjects was not statistically significant.

Data have also been collected for the time judgment (durations of a light on) experiment using the same well educated subjects who participated in the recent size judgment experiments. With this perceptual task, the interference offered by the interpolated task was significantly greater in the old than the young subjects. This result was complicated by a significant age difference in original learning. In order to extract the effects of original learning, a covariance analysis was made. The age difference for re-learning scores remained statistically significant.

The failure of the recent size judgment experiments to support strongly the original hypothesis was at first attributed to the much higher educational level of the subjects used. In the time judgment experiment, however, in which the same high level subjects were used, the results strongly supported the hypothesis. A possible explanation is that there were external cues available in all the size judgment experiments, but it appears that only the

high level subjects made use of them. No such cues were available in the time judgment experiments.

The work described under hypothesis (2) has been completed and is now being written up. Analysis indicates that rate of out-put falls regularly with age regardless of length of order of redundancy; i.e., the main effect, age, is significant, but the second order interactions are not. The third order interactions, age x length x redundancy is significant, however.

Direction of Current Research: No further data will be collected. Publication plans will be handled by the two investigators who initiated these studies. The results, which, in general, support the first hypothesis, are difficult to interpret with solid assurance due to the complex nature of the task. The method employs some of the characteristics of a perceptual judgment study involving discrimination and context effects as well as characteristics of a learning study involving retroaction effects. It would be desirable to design future studies to permit separate evaluations of the contributions of these effects to age differences.

Part B included: Yes

PHS-NIH
Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Suci, G. J.: A comparison of semantic structure in American Southwest culture groups. J. abnormal Psychol., 61: 25-30, July 1960.

Honors and Awards relating to this project:

None

Serial No. NHI-72
1. Gerontology Branch
2. Nutritional Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Age changes in cellular and tissue biochemistry.

Principal Investigator: C. H. Barrows, Jr. (3/4 time)

Other Investigators: N. W. Shock
J. R. Adams (Baltimore City Hospitals trainee)
J. A. Falzone, Jr.

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 4.781
Professional: 1.75
Other: 3.031

Project Description:

Objectives: The general purpose of this program is (1) to examine various tissues of rats for changes associated with senescence in tissue and cellular metabolism and (2) to investigate the effect of diets and pharmacological agents on the concentrations of tissue enzymes as to their potential to reverse changes associated with senescence. Specific problems investigated during this period have been:

(1) The effect of age on protein and fat catabolism.
(2) The effect of age on renal tubular transport estimated in in vitro systems.
(3) The effect of malono nitrile (a compound shown to increase respiratory enzymes, RNA and protein in whole brain cells isolated following administration of the drug, and to reverse peripheral nerve damage) on the concentrations of various enzymes in homogenates of liver, kidney, heart and brain of the rat.
(4) The effect of (a) dietary restriction and (b) dietary proteins during growth on the concentrations of various tissue enzymes.

Methods Employed: (1) Young (12 month) and old (24 month) male rats were injected with S^{35} labeled methionine and C^{14} labeled acetate. One, two, three or four weeks following the injection, the animals were sacrificed and the specific activity of S^{35} in the soluble protein fractions of liver, kidney, heart and muscle was measured. In addition, the specific activity of C^{14} was determined in the fraction obtained by extracting liver and kidney homogenates with chloroform: methanol (3:1) at 60°C for 15 minutes (3X).

(2) Renal cortical slices from 3, 12 and 24 month old female rats were incubated for one hour at 37°C in a modified Krebs Ringer phosphate medium containing 3.88 μg. para-amino hippuric acid (PAH) per ml. Tubular function was estimated by the ratio of the PAH in the slice to that in the medium (S/M) following the incubation period. Analyses for DNA were also made on the same tissue slices.

(3) Eighteen male rats were divided into two equal groups, one of which received 20 mg. of malono nitrile/kg. body weight/day for six days. On the seventh day the animals were sacrificed and the tissues analyzed for the concentrations of various components.

(4a) Eighteen weanling male rats were divided into two groups. One group was fed ad lib. and the other was offered 50% of the amount of food consumed by the former group. At six months of age, the animals were sacrificed and the tissues analyzed for the concentrations of various components.

(4b) Three groups of weanling rats were fed a diet containing one of the following dietary proteins: 20% casein, 32% casein or 20% casein + 12% whole dessicated liver for a period of six weeks. The animals were then sacrificed and the tissues analyzed for the concentrations of various components.

Progress During Past Twelve Months: (1) No significant agewise differences in the rates of catabolism of protein or fat of liver, kidney, heart or muscle were observed.

(2) No age differences were observed in the S/M or in the concentration of DNA in renal cortical slices.

(3) Malono nitrile did not affect the concentrations of succinoxidase, cholinesterase, cathepsin, DNA, RNA or protein nitrogen of the various tissue homogenates. These data suggest that the previous observations may have resulted as a consequence of changes in diffusion on the whole cell preparations rather than an increase in the tissue components per se.

(4a) As would be expected, the body weights and organ weights of the restricted animals were approximately 50% of the normal rats. The concentrations of most of the components of the various tissues did

not differ by more than 10% between the two groups. The exceptions to this were the high succinoxidase of liver (130%) and alkaline phosphatase of kidney (151%) in the restricted animals.

(4b) These data demonstrated an increased concentration of liver pyrophosphatase in the groups receiving the higher amounts of protein and an increased liver d-amino acid oxidase in the group receiving the whole dessicated liver.

The last two studies (4a, 4b) demonstrate that the marked retardation of growth accompanying dietary restriction, which has been shown to increase the life span of rats, results in alterations in the concentrations of certain tissue enzymes. Furthermore, they indicate that it is possible to affect the concentrations of some enzymes in growing animals by feeding diets which will promote optimum growth but differ in the quality or quantity of the dietary protein. Thus it may be possible to choose a diet which will support normal growth and still increase the life span.

Direction of Current Research: (1) Future experiments will include feeding animals various diets which are believed to result in biochemical differences within the tissues, with the ultimate goal of determining the effectiveness of the diets in increasing longevity or maintaining youthful vigor in the senescent rat.

(2) Studies on oxidative phosphorylation of mitochondria isolated from liver will be continued until an adequate method for the measurement of the rates and efficiency in animals of different ages can be obtained.

Part B included: Yes

PHS-NIH
Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Barrows, C. H., Jr., J. A. Falzone, Jr., and N. W. Shock: Age differences in the succinoxidase activity of homogenates and mitochondria from the livers and kidneys of rats. J. Geront., 15: (2), 130-133, April 1960.

2. Barrows, C. H., Jr.: Age and cellular metabolism of tissues. In: B. L. Strehler (Editor), The Biology of Aging. American Institute of Biological Sciences, Washington, 1960, Publ. #6, pp. 116-118.

Honors and Awards relating to this project:

Serial No. NHI-73
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Age changes in human physiological performance

Principal Investigator: A. H. Norris (1/3 time)

Other Investigators: N. W. Shock
J. A. Falzone, Jr.

Cooperating Units: Baltimore City Hospitals

Dr. Robert W. Ramsey, Medical College of Virginia, Richmond, Virginia

Dr. John Magladery, The Johns Hopkins University, School of Medicine, Baltimore, Maryland

Dr. Robert D. Teasdall, The Johns Hopkins University, School of Medicine, Baltimore, Maryland

Man Years (1960):

Total:	3.333
Professional:	0.333
Other:	3.000

Project Description:

Objectives: This project is designed to study the effects of aging on (a) the physiologic responses to exercise, (b) the rate of recovery of physiologic equilibrium after exercise, (c) muscular efficiency and (d) work output and fatigue. In addition, the factors responsible for limitations in performance observed in older people will be evaluated.

Methods Employed: Measured amounts of physical work are obtained in subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake, CO_2 elimination, pulmonary ventilation volume, heart rate, blood

pressure, and cardiac output (by the dye method) are taken before, during, and after standardized amounts of exercise. In addition, the volume of the lungs and functional capacities of the pulmonary system are evaluated. Other studies include measurements of speed of nerve conduction, reflex delay time, and muscle action potentials. These phenomena are recorded on a six channel oscillograph or dual beam oscilloscope as the experiment demands.

Progress During Past Twelve Months: In cooperation with Dr. Ramsey and co-workers we have developed a description of the mechanical relationships of a human arm swinging maximally in alternating back and forth movements at different specified amplitudes. During this maximal wagging exercise there was a linear relationship between (1) period of swing and angular displacement (amplitude of swing), (2) the impulse of the force applied to the limb and the log of the angular displacement, and (3) action and angular displacement. Moreover, it was found that none of these relationships was related to mechanical efficiency derived from measurements of the oxygen cost of the work performed. Nevertheless, a family of curves was developed which related mechanical efficiency to age at different amplitudes of swing so that young subjects (30 years old) increased efficiency with increase in amplitudes of swing while old subjects (70 years old) did not increase efficiency with increase in amplitude of swing. In addition, in both old and young subjects, the angular proportion of the swing during which there was force exerted on the limb by the muscles was found to decrease (93% to 57%) with increase in amplitude (0.36 Rad. to 1.85 Rad.).

Direction of Current Research: Measurements of the effects of arm loading will be continued for a single amplitude of arm swinging. In addition, information transfer expressed as the error function of this and/or similar movements will be evaluated.

Comparison of muscle strength and maximal work output (arm cranking exercise) will be continued with metabolic measurements. In addition, corrections for the oxygen cost of breathing work will be made.

Part B included: Yes

PHS-NIH
Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Norris, A. H., and N. W. Shock: Exercise in the adult years--with special reference to the advanced years. Chap. 24, in: W. R. Johnson (Editor), Science and Medicine of Exercise and Sports. Harper & Bros., New York, 1960, pp. 466-490.

2. Hägin, F., A. H. Norris, and N. W. Shock: Skin reflex and voluntary reaction times in young and old males. J. Geront., 15: 388-391, October 1960.

3. Baker, S. P., M. Landowne, and G. W. Gaffney: Electrocardiographic changes following the administration of thyroid stimulating hormone (thyrotropin). Amer. J. Cardiol., 6: (5), 905-914, Nov. 1960.

Serial No. NHI- 74
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Age differences in body size and composition

Principal Investigators: A. H. Norris (1/3 time)
T. H. Lundy (Full time - resigned 6/30/60)

Other Investigators: N. W. Shock
M. J. Yiengst

Cooperating Units: The Baltimore City Hospitals

Dr. Harald Schraer, The Bone Density Research and Evaluation Center, Pennsylvania State University, University Park, Pennsylvania

Dr. Stanley M. Garn, Fels Research Institute, Antioch College, Yellow Springs, Ohio

Dr. Saul P. Baker, Department of Medicine, Chicago Medical School, Chicago, Illinois

Man Years (1960):

Total:	2.341
Professional:	0.883
Other:	1.458

Project Description:

Objectives: This project is designed to describe age differences in body size and composition, to compare various size and composition measures made concurrently in individual subjects, and to examine the relationship of these age differences and comparisons to physiological responses.

Methods Employed: Height and weight data will be obtained by the usual anthropometric methods. The volume of the body will be measured by its displacement of helium in a closed chamber. The density of the body will be calculated from these measures after correction for total skeletal mass estimated from roentgenographic films of large and small bones. Body fat will also be estimated

from skinfold thickness and roentgenographic techniques. Estimates of lean body mass will include (1) basal metabolic rate determinations made with standard open circuit methods, (2) urinary excretion of creatinine, and (3) body water and fluid distribution determinations made from the distribution curves of injected antipyrine and sodium thiocyanate. This is a continuing program. These studies will be carried out in people of different ages and in the same people as they become older.

Progress During Past Twelve Months: Although our longitudinal studies subjects were heavy according to the standard weight tables, there was no significant age difference between old (70-79 years) and young (30-39 years) subjects. Total body density, total body water, and extracellular water did not show significant age differences, while there were significant differences between old and young for creatinine excretion, basal oxygen consumption and the density of the middle phalanx of the small finger. Lean body mass and fat were calculated from equations proposed by Dr. Keys, Dr. Siri, and Dr. Lundy. Dr. Keys' equation related individual values to ideal body composition of young subjects and makes appropriate corrections for composition of tissues presumed to be gained or lost. The Lundy equation adds to Keys' equation corrections for what we know about age differences in (1) muscle mass as measured by creatinine excretion and (2) bone density from the phalangeal measurements. The Siri equation combines estimates of total body density and body water to reduce the variance in the resulting calculations of lean body mass and fat. When fat calculated from the Keys' equation and expressed as a percentage of body weight was plotted against age, a slight increase of 0.06% fat per year was found. However, when fat was calculated by the Lundy equation and similarly expressed, there was a decrease of 0.12% per year. The probability of a difference between these two slopes was (P = less than 0.025). This difference in the fat trend results from the use of estimates of bone and muscle changes rather than making assumptions about them.

Direction of Current Research: The integrated measurement program which includes selected indices of body size and composition will be continued. Some techniques are provided through cooperation of investigators outside of the Public Health Service while others are standardized procedures used in this laboratory. Data are available for comparison with other physiological data which may be collected on subjects of these studies. Moreover, subjects whose size or composition varies widely from mean values will be selected for study, and experimental and therapeutic displacements of body composition may be attempted.

Part B included: No

Serial No. NHI-75
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Pulmonary physiology as related to age

This project has been combined with NHI-73: Age changes in human physiological performance.

Serial No. NHI- 76
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Longitudinal studies of human physiology, biochemistry and psychology.

Principal Investigators: N. W. Shock (Director)
A. H. Norris (Coordinator) (1/3 time)
J. A. Falzone, Jr. (Clinical Director) (1/4 time)
J. S. Dodd (1/2 time - E.O.D. 7/1/60)

Other Investigators: M. D. Davidoff
M. J. Yiengst
W. W. Surwillo
D. Arenberg
R. B. McGandy
T. Lundy
J. Lowenstein
A. S. Mildvan
D. A. Faulstick
F. F. Johnston
H. Langs

Cooperating Units: Baltimore City Hospitals
Maryland State Health Department
Dr. Bernice Cohen, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland
Miss Marjorie Cantoni, Heart Disease Control Program, U.S. Public Health Service, Washington, D. C.

Man Years (1960):

Total:	8.33
Professional:	3.33
Others:	5.0

Project Description:

Objectives: This project is designed to (1) facilitate repeated measurements of various physiological, biochemical and psychological variables in the same individuals as they grow older, (2) coordinate the utilization of the results of these measurements in establishing indices of aging and (3) compare the effects of socio-economic status on these results and the indices which may be derived therefrom.

Methods Employed: Participants are recruited by other participants. They agree to be available for a three-day series of tests every 18 months for the rest of their lives (agreement in principle--there are no binding articles). A given series of tests will be repeated at a five year interval. Measurements of body size and composition, pulmonary function, kidney function, psychological function, neuromuscular function and cardiovascular function are made. Physical examinations and medical histories are performed with check lists to assure uniformity of recording. Reports of medical findings and pertinent test results are made to a participant's private physician only. Participants are treated as a group (named The Six S's, which stands for Select Society of Seeking Scientists, Saints and Sinners) and kept informed of the progress of the total program and of test results for the group. Data are kept in restricted files and are summarized by calculating machine methods.

Progress During Past Twelve Months: During this period our interests have been expanded to include diet and nutrition and human genetics. In the area of nutrition, a specific proposal has been made by Dr. McGandy to facilitate a survey of the dietary habits of the participants in this study. These results will be compared with measurements of body composition which are also being made in these subjects. In the area of human genetics, Dr. Cohen has begun collecting data from available participants which should shed light on the question of assortative mating with relation to longevity background. In the psychological area, Dr. Arenberg has begun new studies of the effects of aging on learning processes.

Direction of Current Research: The two series of tests which are now in progress will be continued. An additional series of tests will be begun in the spring of 1961. Results will be summarized and evaluated as they become available. In addition, methods of data handling are being examined to determine what will be appropriate and efficient for these studies.

Part B included: Yes

PHS-NIH
Individual Project Report
1960

Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Norris, A. H., and N. W. Shock: The Six S's. Newsletter (Geront. Soc.), 7: (1), 7, March 1960.

2. McCarthy, J. D. (Moderator), N. W. Shock, F. C. Swartz, and J. Weinberg (Panelists): Panel discussion on concepts of the medical profession in relation to aging. J. Amer. geriat. Soc., 8: (8), 591-603, August 1960.

3. Shock, N. W.: Age changes in physiological functions in the total animal: the role of tissue loss. In: B. L. Strehler (Editor), The Biology of Aging. American Institute of Biological Sciences, Washington, 1960, Publ. #6, pp. 258-264.

4. Shock, N. W.: Some of the facts of aging. In: N. W. Shock (Editor), Aging: Some Social and Biological Aspects. American Association for the Advancement of Science, Washington, 1960, Publ. #65, pp. 241-260.

Honors and Awards relating to this project:

Dr. Shock received the 1960 Modern Medicine Award for Distinguished Achievement for contributions to the progress of medicine.

Serial No. NHI-77
1. Gerontology Branch
2. Molecular Biology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Structure of hemoproteins.

Principal Investigator: Gunther L. Eichhorn (1/3 time)

Other Investigator: Wilbur W. Everett (1/6 time)

Cooperating Units: Baltimore City Hospitals

Man Years (1960):

Total:	1.196
Professional:	0.499
Other:	0.697

Project Description:

Objectives: (a) To elucidate the nature of the relationship between protein and prosthetic groups in such compounds as hemoglobin, cytochrome-C, etc.

(b) To evaluate the relationship between the structures of these compounds and their rotatory dispersion curves.

Methods Employed: Samples of various biologically important hemoproteins are prepared, and their absorption spectra and rotatory dispersion curves are determined. If the rotatory dispersion is anomalous in regions where the heme absorbs, the heme group must be an asymmetric center. It is then possible to subject the molecules to various chemical stresses, and to determine the effect upon the asymmetric center.

Progress During Past Twelve Months: A correlation of the rotatory dispersion curves of a variety of hemoproteins with other physical properties under various conditions has led to the following generalization: the Cotton effect associated with the heme absorption is a measure of the ordered arrangement of the protein, and disappears when the structure of the protein has been destroyed.

The best evidence comes from a comparison of catalase with peroxidase. Both of these proteins exhibit strong Cotton effects in their natural state at neutral pH, but lose the effect in acid and basic solutions. In catalase the phenomenon could be explained by the splitting of the four-heme molecule into quarters that contain only one heme. Peroxidase, however, contains only one heme, and cannot be split into smaller fragments; this was shown by ultracentrifugation experiments. The loss of the Cotton effect must therefore be due to denaturation.

It has been shown that the stability of the heme-attaching site in catalase is much greater than the stability of the molecule as a whole. When the heme is detached from catalase, an apocatalase is produced which will react with four moles of heme to form a "reconstituted catalase" with many physical properties similar to those of intact catalase, but with no Cotton effect. The stoichiometry of the reaction attests to the stability of the heme site and the loss of Cotton effect to the instability of the structure of the protein as a whole.

Further studies with hemoglobin and myoglobin have revealed that the origin of the Cotton effect in these molecules is the same as in catalase and peroxidase; the heat denaturation of hemoglobin has been studied by rotatory dispersion.

Direction of Current Research: Experiments are under way to refine some of the existing data. Otherwise, it is considered that this project has been brought to conclusion.

Part B included: Yes.

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Part B Honors, Awards and Publications

Publications other than abstracts from this project:

None

Honors and Awards relating to this subject:

1. Gunther Eichhorn was one of thirty scientists invited to participate at a Conference on Optical Rotation at Rancho Santa Fe, California, January, 1960. The manuscript presented, "Rotatory Dispersion Studies of Biologically Active Metalloporphyrins" is in the process of publication by Pergamon Press.

Serial No. NHI-78
1. Gerontology Branch
2. Molecular Biology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Structure of nucleic acids.

Principal Investigators: Gunther L. Eichhorn (1/3 time)
Wayne W. Everett (1/6 time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):

Total:	1.165
Professional:	0.499
Other:	0.666

Project Description:

Objectives: (1) To find a method of determining terminal groups in polynucleotides; (2) To introduce a new technique for sequence studies in nucleic acids; and (3) To study the reaction of polynucleotides with the metal complexes of hydroxyaldehydes.

Methods Employed: Three of the four nucleotide bases of DNA and RNA have amino groups, which can be made to react with salicylaldehyde and metal ions to form complexes of widely varying stabilities. It is hoped that this reaction will distinguish between the four bases at terminal positions of a polynucleotide.

Varying quantities of metal ions and salicylaldehyde will be reacted with the nucleic acids; it is hoped that this reaction will create weak links in the polynucleotide chain; subsequent hydrolysis will then cleave the nucleic acid at specific points, in a manner resembling that of proteolytic enzymes.

Organic dialdehydes will be constructed with their aldehyde functions the correct distance apart for reaction with both amino groups of any given

sequence in polynucleotides. Hydrolysis of the polynucleotides will then reveal the sequences that actually exist; correlation of the resulting information with enzymatic sequence studies should produce much longer nucleotide sequences than any that have been obtained so far.

Progress During Past Twelve Months: Research activity in this section has been concentrated on this project, especially in the latter period, because of the discovery of the remarkably specific reactions that can be achieved with the various nucleic acid degradation products by complex formation with cobalt, copper, cobalt salicylaldehyde and copper salicylaldehyde. By a combination of such reactions, using dimethyl sulfoxide as solvent, color tests have been developed to distinguish qualitatively with much greater simplicity and specificity than heretofore possible, between the following substances: ribose, deoxyribose, adenine, cytosine, guanine, uracil, thymine, adenosine, cytidine, guanidine, deoxyadenosine, deoxyguanidine, deoxycytidine, as well as the nucleoside phosphates and deoxynucleoside phosphates. These methods are presently being developed for quantitation.

The reaction of nickel-salicylaldehyde, cobalt-salicylaldehyde, and copper-salicylaldehyde with the four nucleotide bases gave the expected specificity by producing a different complex with adenine than with the other three. In the nucleosides and the nucleotides the metal reacts also with the ribose moiety of these substances, thus decreasing the specificity of the reaction.

Direction of Current Research: The substitution of pyridine-2-carboxaldehyde for salicylaldehyde is presently being investigated. A chromatographic scheme for the separation of cleavage products has been completed. Various polynucleotides will be allowed to react with cobalt, nickel, and copper complexes of salicylaldehyde and pyridine-2-carboxaldehyde to determine whether end groups can be made to react preferentially, and whether it is possible to utilize these reactions as cleavage agents for specific nucleotide linkages.

Part B included: No

Serial No. NHI- 79
1. Gerontology Branch
2. Molecular Biology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: The function of metal ions in enzymatic reactions.

Principal Investigator: Gunther L. Eichhorn (1/3 time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 0.51
Professional: 0.51
Other: None

Project Description:

Objectives: To arrive at an understanding of the mechanism by which metal ions participate in enzymatic reactions, and also to determine why different metal ions are required for different enzymatic processes.

Methods Employed: It is assumed that in any metal-catalyzed enzymatic process the metal ion must serve either (1) as an active site, by direct attachment to the substrate, or (2) it must react with the enzyme protein, and thus alter the secondary and tertiary structure of the latter, producing the active configuration. It is postulated that, whenever scheme (1) applies, the metal should be capable of bringing about a non-enzymatic reaction; failure of the non-enzymatic reaction, inversely, would favor scheme (2).

Specially selected enzymatic substrates are treated with metal ions under varying conditions, and the nature of the metal-substrate interactions detected by physical measurements, e.g., spectrophotometry. The interaction of the metals with the enzyme protein is studied by macromolecular techniques, such as ultracentrifugation.

Progress During Past Twelve Months: An examination of metal-activated enzymatic reactions was carried out to explain the fact that the order of effectiveness of the various metals differs from enzyme to enzyme, and is generally different from the order of effectiveness of metals in non-enzymatic catalysis. The discrepancies were explained by the fact that the order may be the same in enzymatic as well as non-enzymatic reactions, but that this order applies in enzyme inhibition. Thus the effectiveness of a metal in any enzymatic reaction depends upon a compromise between ability to catalyze the reaction and yet to be minimally destructive to the enzyme.

The studies on the effect of metals on the aconitase substrates were concluded by a determination of the magnetic susceptibilities of the metal complexes of these substrates. It was found that all of the complexes have identical magnetic moments, and concluded that the difference in the abilities of the various metal ions to catalyze the aconitase reaction is probably not due to thermodynamic stability differences, but should be ascribed to kinetic effects.

Metal-protein interactions are discussed in project NHI-46.

Direction of Current Research: Enolase will be prepared for rotatory dispersion and sedimentation studies of the enzyme and its metal complexes. The effect of metals on enolase will be studied by ultracentrifugation and optical rotation techniques.

Part B included: Yes

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Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Eichhorn, G. L.; The role of metal ions in enzyme systems. Chap. 2, in: M. J. Seven (Editor), Metal Binding in Medicine. J. B. Lippincott Co., Philadelphia, 1960, pp. 19-26.

Honors and Awards relating to this project:

1. Gunther Eichhorn was asked by the American Chemical Society to organize a Symposium on the "Role of Metal Ions in Biological Processes," scheduled September 12-13, 1960, in New York.

2. Gunther Eichhorn was invited to present one of four lectures on the Physical Chemistry of Chelation at a Conference on the Biological Aspects of Metal Binding, sponsored by the National Academy of Sciences at University Park, Pa., September 6-9, 1960.

3. Gunther Eichhorn has been asked to contribute a chapter to a revised edition of "The Chemistry of the Coordination Compounds," edited by J. C. Bailar, Jr.

4. Gunther Eichhorn has been invited to present a paper at a symposium on Trace Metals before the New York Academy of Science, March, 1961.

5. Gunther Eichhorn has been invited to present a paper at the International Conference on Coordination Chemistry, Detroit, September, 1961.

Serial No. NHI-80
1. Gerontology Branch
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Action of steroids and sterols at the cell level.

Principal Investigator: D. E. Buetow (50% time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 1.60
Professional: 0.50
Other: 1.10

Project Description:

Progress During Past Twelve Months: Cultures of a streptomycin-bleached Euglena were grown in the presence and absence of testosterone. The cells were harvested during the logarithmic growth phase, concentrated by centrifugation, and washed twice and resuspended in medium lacking testosterone. Cells prepared in this fashion showed the same QO_2 regardless of whether they had previously been grown in the presence or absence of testosterone. However, when testosterone was added to the substrate for respiration, there was an increased QO_2 over the control. This increase was the same regardless of the previous growth condition used. The higher QO_2 of Euglena in the presence of testosterone appears to be dependent on the presence of a nitrogen source in the respiration medium. If nitrogen is absent from the medium, the QO_2 of Euglena in the presence of testosterone is the same as in its absence. In these experiments, concentrations of 0.1 or 2.0 μg. testosterone per ml. of medium were used. Past work has also shown an effect of testosterone on the dry weight and protein content of these cells.

Cultures of these same streptomycin-bleached Euglena were also grown in the presence of vitamin D_3 or ergosterol in concentrations ranging from 1.0 μg. to 10^{-3} μg. per ml. of medium. Cultures containing vitamin D_3 at a concentration of 10^{-2} μg/ml. show an increased growth rate over control cultures. Ergosterol at this concentration does not affect the growth rate of Euglena.

Direction of Current Research: A continuous culturing apparatus has been designed and will be used to maintain cultures at a constant density. Radioactive calcium will be used to determine whether or not Euglena concentrates calcium in the presence of vitamin D_3. The respiration of Euglena in the presence and absence of vitamin D_3 is currently being investigated.

Radioactive testosterone and/or nitrogen will be used to gain insight into the problem of whether this steroid acts in conjunction with specific enzymes or acts directly on permeability. Mitotic indices, size distributions and RNA and DNA content of Euglena grown in the presence and absence of testosterone are currently being investigated.

Part B included: No

Serial No. NHI-81
1. Gerontology Branch
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Effects of age on single cell cultures.

Principal Investigators: D. E. Buetow (50% time)
J. J. Blum (25% time)

Other Investigator: None

Cooperating Units: Baltimore City Hospitals

Man Years (1960):
Total: 0.95
Professional: 0.75
Other: 0.20

Project Description:

Progress During Past Twelve Months: It has now been shown that some protozoa can be maintained for many months in the absence of a carbon source or of a nitrogen source from the medium. During this prolonged period of starvation--aging, the cells become small, fairly transparent, and relatively non-motile. Their content of RNA greatly decreases. When returned to a complete medium, they exhibit a lag period before resuming growth. The longer the cells have been exposed to the nitrogen or carbon deficient medium, the longer is the lag period. Cells which are deprived of sulfur have been found to die within one month, thus marking the lack of sulfur as more critical than the lack of either carbon or nitrogen.

Direction of Current Research: We are presently measuring the DNA content of these cells at various times of "aging" to see whether there is any change in this substituent. After this point is clarified, we hope to study control mechanisms concerned with adjusting the cell to its carbon deficient environment.

Part B included: No

Serial No. NHI-82
1. Gerontology Branch
2. Biophysics Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: The time of synthesis of some protein and lipid fractions in cultures of synchronously dividing cells.

Principal Investigator: G. Padilla (100% time - E.O.D. 10/15/60)

Other Investigators: J. J. Blum
D. E. Buetow

Cooperating Units: Baltimore City Hospitals and the Division of Research Grants and Fellowships, National Institutes of Health

Man Years (1960):
Total: 0.208
Professional: 0.208
Other: None

Project Description:

Progress During Past Twelve Months: None. This is a new project.

Direction of Current Research: Before coming here on his post-doctoral fellowship, Dr. Padilla worked out a technique for synchronizing the mitotic pattern of protozoa grown in a well defined synthetic medium. We intend to use radioactive sulfur and acetate to trace the synthesis of any protein and lipid fractions that are synthesized just prior to mitosis.

Part B included: No

Serial No. NHI-83
1. Gerontology Branch
2. Physiology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
1960

Part A

Project Title: Human Nutrition Studies: Nutrition survey in the longitudinal study.

Principal Investigator: R. B. McGandy (1/3 time - E.O.D. 6/20/60)

Other Investigators: N. W. Shock
A. H. Norris

Cooperating Units: Maryland State Health Department
Miss Marjorie Cantoni, Heart Disease Control Program, U. S. Public Health Service, Washington, D.C.
Baltimore City Hospitals

Man Years (1960):

Total:	0.155
Professional:	0.155
Others:	None

Project Description:

Objectives: (1) To characterize dietary intakes and patterns of eating in the Longitudinal Study Group and to describe age-related differences within the group.

(2) To correlate age-related differences with various pertinent morphologic and physiologic measurements.

(3) To describe changes in dietary intakes and patterns of eating as a function of time and to relate these changes to alterations in morphologic and physiologic measurements during the same period.

Methods Employed: For the purpose of surveying the dietary intakes and eating patterns, a seven day diet record form will be kept by subjects in the Longitudinal Study who have had the medical screening examination. To

assess seasonal and other variability, three such records will be collected on each man during the year. A nutritionist will assist the subjects by instructing them in how to keep the record. As time permits, some subjects will be used to test the adequacy and validity of the record of estimated intakes by a comparison with weighed intakes of the various dietary components. Also, through interviews, estimates of long term trends in eating habits will be obtained.

The data thus collected will be analyzed for the purpose of characterizing the types of foods eaten as well as for quantitation of specific nutrients by the methods in use at the National Heart Institute. Correlations between nutrient data and information already being collected in this study on such parameters as body composition, estimates of energy expenditure, and plasma cholesterol and hemoglobin levels will be performed.

Progress During Past Twelve Months: This is a new program.

Direction of Current Research: The various forms for collection of the dietary information are being developed. Also methods for handling the correlation analyses will be developed.

Part B included: No

Serial No. NHI-84
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Transport Function of the Atrium. Factors Influencing the Relation between Mean Atrial Pressure and Ventricular End Diastolic Pressure.

Principal Investigators: J. H. Mitchell, S. J. Sarnoff, J. P. Gilmore

Other Investigators: None
Technicians: F. Perry, C. Scott, D. Edwards, C. Whitted
Cooperating Units: None

Man Years (calendar year 1960):

Total:	1.61
Professional:	.65
Other:	.86

Project Description:

Objectives - Methods Employed: The pressure in the atrium not only provides for the progress of blood into the ventricle but is also the central pressure head which must be exceeded by the pressure of the blood in the veins feeding into it. From the point of view of integrating the heart with the total organism it is, therefore, pertinent to inquire whether a change in the activity of the atrium modifies the mean pressure in it for any given end diastolic pressure, stroke work, and cardiac output.

Measurements were made of mean left atrial pressure, left ventricular end diastolic pressure, cardiac output and heart rate.

Major Findings:

(1) When changing from atrial to ventricular pacing such that the atrial contribution to ventricular filling was precluded by a closed mitral valve, mean atrial pressure rose, and left ventricular end diastolic pressure, stroke volume and stroke work fell. Thus when changing from atrial to ventricular pacing, mean atrial pressure always became higher in relation to left ventricular end diastolic pressure.

(2) As heart rate was increased mean atrial pressure became higher in relation to left ventricular end diastolic pressure.

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(3) During cardiac sympathetic nerve stimulation the mean left atrial pressure is lower for any given left ventricular end diastolic pressure than in the control study. Conversely, during efferent vagal nerve stimulation the mean left atrial pressure is higher than in the control study. Changes in atrial contractility contribute substantially to these altered relations. At any given heart rate during cardiac sympathetic nerve stimulation the atrium contracts more vigorously and empties more completely. This decreases residual atrial volume and places the atrium on a lower portion of its pressure-volume curve so that for any given ΔV it requires a smaller ΔP. Conversely, during efferent vagal nerve stimulation atrial contractility is decreased and emptying is less complete. Therefore, residual atrial volume is increased, and the atrium is placed on a higher portion of the pressure volume curve, thus requiring a greater ΔP for any ΔV. Further, the shorter the time required for atrial systole, the longer the period during which the atrium will be relaxed and will, therefore, be more distensible.

Whereas, the relation between ventricular end diastolic pressure and stroke work is determined only by the performance characteristics of the ventricle, the relation between mean atrial pressure and stroke work is determined by the performance characteristics of the atrium as well as the ventricle. Thus, the importance of a normally functioning atrium invites consideration of the possibility that, when an elevated venous pressure is observed, failure of the atrium as well as the ventricle may be a contributing factor.

Proposed Course of Project: Project completed. Manuscript in preparation.

Part B included: No

Serial No. NHI-85
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Influence of Atrial Activity on Mitral Valve Closure.
Principal Investigator: S. J. Sarnoff

Other Investigators: J. H. Mitchell and J. P. Gilmore
Technicians: F. Perry, D. Edwards, C. Whitted
Cooperating Units: None

Man Years (calendar year 1960):

Total	1.26
Professional	.65
Other	.61

Project Description:

Objectives - Methods Employed: This investigation was aimed at examining the possibility that atrial activity can produce mitral valve closure.

The open-chest, anesthetized dog with surgically induced heart block was employed. Pressures were recorded in left atrium, left ventricle and aorta.

Major Findings:

1. The mitral valve can be closed solely as the result of atrial activity as evidenced by the sustained plateau of ventricular diastolic pressure following the peak of each atrial systole at a time when atrial pressure declines. Ventriculo-atrial pressure gradients of over 8 cm. H_2O were observed.

2. When the pressure pattern in the left ventricle was such as to indicate that mitral valve closure was taking place, this could be abolished by partially inhibiting atrial activity with vagal stimulation. When, in the control period, the ventricular pressure pattern indicated that mitral valve closure was not taking place, this could not infrequently be induced by sympathetic stimulation.

3. The above observed effects on the activity of the atrium and the resultant presence or absence of mitral valve closure could be

reflexly manipulated as, for example, by raising or lowering carotid sinus pressure.

4. It was concluded that the amplitude of atrial systole can substantially influence whether or not mitral valve closure occurs prior to the onset of ventricular systole. These findings are consonant with the clinical observation that a systolic murmur will sometimes occur with the onset and disappear with the cessation of atrial fibrillation.

Proposed Course of Project: Project completed. Manuscript in preparation.

Part B included: No

Serial No. NHI-86
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Norepinephrine and Epinephrine Estimation in Plasma

Principal Investigator: S. J. Sarnoff

Other Investigators: J. Cox
Technicians: M. Edmunds
Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.20
Professional: .20
Other: 1.00

Project Description:

Objectives - Methods Employed: In the early stages of the study the fluorometric method of Weil-Malherbe (EDA) was employed for the measurement of catechol amines (N & E) changes in coronary arterial and venous blood. At the time this method appeared to be the best suited for such measurements. However, during the course of the cardiac sympathetic experiments it was observed that although the EDA method showed an increase in EDA condensing material in coronary venous blood during cardiac sympathetic nerve stimulation, there frequently appeared in coronary venous blood in the post stimulatory period, an increased concentration over the control coronary venous levels of a substance with the same fluorescent peak as norepinephrine. Since this post stimulatory "norepinephrine" was not associated with any of the hemodynamic changes characteristic of the response to catechol amines, the Weil-Malherbe assay was compared with the Trihydroxyindole (THI) method as modified by Crout. These comparisons have shown that the catechol amine concentration of coronary venous blood during cardiac sympathetic nerve stimulation as determined by the THI assay, parallel the hemodynamic changes in the dog. The catechol amine values obtained on the same samples by the EDA method show no such parallelism. In almost all instances the EDA condensing material was greater than the catechol amine values found by the THI method. In some experiments the ethylenediamine condensing material in excess of the total catechol amines measured by the THI method was analysed chromatographically by Dr. J. R. Crout, and was found not to be retained by a cationic exchange resin and was therefore clearly demonstrated not to be a catechol amine. However, there remains the

possibility that either a neutral catechol or a catechol acid may account for the difference.

On the basis of these findings, it would appear that previous studies of plasma catechol amines and their hemodynamic relationships as determined by variants of the EDA method would require reinterpretation. The results of using the THI method of analysis yield what appear to be consistently interpretable results both as regards the analysis of "unknowns" and the data obtained from in vivo sampling in varying states.

Proposed Course of Project: It is felt that the THI technique is satisfactory for the purposes at hand and will continue to be used as the method of choice in this laboratory.

Part B included: No

Serial No. NHI-87
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effect of Stellate Stimulation on Arterial Pressure, Atrial Pressure and Renal Function.

Principal Investigator: J. P. Gilmore

Other Investigators: None
Technicians: J. Miles

Man Years (calendar year 1960):
Total: .75
Professional: .25
Other: .50

Project Description:

Objectives - Methods Employed: In the initial aspects of the study it was observed that stimulation of the stellate ganglion of the dog produced an immediate diuresis independent of changes in mean arterial pressure and accompanied by an increase in glomerular filtration rate (GFR). It was also observed that in these dogs which received little or no fluid during the experiments, no change in urine $[K^+]$, $[Cl]$ or osmolarity occurred during the diuresis, and that vagotomy significantly diminished the extent of the diuresis. It was suggested that "stellate diuresis" was the result of a reflex diminution of sympathetic activity to the kidney which causes an increase in the effective filtration pressure.

Major Findings:
Recent experiments have shown:

(1) That stellate diuresis also occurs in the dog during mannitol (5%) diuresis. However, during diuresis under these conditions urinary $[Na^+]$ increases. It was also observed that as urine flow increases as the result of the mannitol infusion, $[\dot{U}_{Na^+}]$ increases while urine osmolarity decreases.

(2) The renal response to carotid occlusion is quite variable from dog to dog. In some animals carotid occlusion increased, GFR and urine flow, while in others although GFR showed little change, urine flow fell. Because of the variability in the response prior to vagotomy, no definite pattern has yet been seen in response to carotid occlusion subsequent to bilateral vagotomy. In an attempt to control

some of the variables a preparation is being devised in which it will be possible to have a completely innervated kidney in which pressure and/or flow can be controlled.

Proposed Course of Project: To continue investigation of renal function during increased (carotid occlusion) and decreased (stellate stimulation) sympathetic activity using a flow and/or pressure controlled innervated kidney and to test the hypothesis that infusion diuresis is attributable at least in part to the stellate diuresis mechanism.

Part B included: No

Serial No. NHI-88
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effects of Carotid Body Stimulation on Cardiovascular Dynamics.

Principal Investigators: J. H. Mitchell, J. P. Remensnyder and S. E. Downing

Other Investigators: None
Technicians: F. Perry, C. Scott, C. Whitted

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.40
Professional: .70
Other: .70

Project Description:

Objectives: To study the effects of graded changes in pO_2 and pCO_2 of blood perfusing the isolated carotid bodies on heart rate, myocardial contractility and peripheral vascular resistance.

Methods Employed: Pressures are measured in the aorta, left atrium, left ventricle and the isolated carotid sinuses. Aortic flow is monitored by a Potter electroturbinometer.

The blood perfusing the isolated carotid sinus and body region is equilibrated with various gas mixtures by a disc oxygenator. The changes in pO_2 are continuously monitored by a Clark polarographic electrode and pH changes by a Beckman electrode; pCO_2 is calculated from the determination of pH and CO_2 (Van Slyke).

Heart rate is either allowed to vary or is controlled by atrial pacing. Perfusion pressure in the carotid sinus is controlled by a Dale-Schuster pump.

A method of vagal nerve cooling, for reversible blockade of the vagus, is being developed for this project.

Major Findings: Hypoxia and hyperpnea cause a substantial bradycardia before section of the vagus nerves. It also appears that

hypoxia and hyperpnea cause an increased myocardial contractility after vagotomy but satisfactory studies prior to vagotomy have not yet been attained.

Proposed Course of Project: To investigate the effects of carotid body stimulation on heart rate and myocardial contractility before and after section of the vagus nerve, and also to study changes in peripheral vascular resistance and perhaps venous tone.

Part B included: No

Serial No. NHI-89
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: An Investigation of the Effects of Afferent Activity from the Carotid Sinus and Carotid Body upon Sympathetic Efferent Discharge to the Heart in the Cat..

Principal Investigators: S. E. Downing, J. H. Siegel

Other Investigators: None
Technicians: C. Whitted

Man Years (calendar year 1960):
Total: .75
Professional: .50
Other: .25

Project Description:

Objectives: It has been shown that afferent activity from the carotid sinus (input) may modify the sympathetic efferent discharge (output) from the cardiac centers. An evaluation of certain other factors which may alter sympathetic discharge to the heart was undertaken. The purpose of this investigation is: 1) to determine by direct measurement the effect of carotid body and carotid sinus activity upon sympathetic discharge to the heart; 2) to evaluate the significance of the gas composition of the blood perfusing the medullary cardiac centers upon sympathetic discharge to the heart; and 3) to determine the interrelationships between the above factors.

Methods Employed: The efferent discharge to the heart is measured by recording nerve impulse traffic from the lesser cardiac nerve using standard electroneurographic recording techniques. The left carotid sinus region is vascularly isolated and separately perfused utilizing apparatus which permits wide variation of perfusion pressure, static or pulsatile, and rapid change from fully saturated to desaturated blood. The right carotid sinus nerve and the cervical vagi are cut. Therefore, the input to the cardiac centers from the cardiac sensory areas is assumed to be largely restricted to the left carotid region. The cat is respired mechanically, though the chest is not opened, to keep the systemic blood pH and gas composition constant. By introducing gases of known composition into the respirator the pH and blood gas composition of the cardiac centers may be altered over a wide range.

Major Findings: The project is in its early stages. However, the following observations have been made: 1) The application of a pulsatile pressure to the carotid sinus produces a synchronous pulsatile discharge from the lesser cardiac nerve; 2) when the pulsatile input is stopped, the pulsatile discharge continues for 3-4 seconds; 3) a rapid rise in endosinus pressure produces a marked reduction or cessation of sympathetic discharge for a brief period; 4) the introduction of markedly desaturated blood into the isolated carotid sinus appears to cause reduction of tone in the vessel wall; this is reversed by the reintroduction of saturated blood.

Proposed Course of the Project: It is proposed to continue with these investigations in an attempt to evaluate the importance of input data to the medullary cardiac centers in modifying or controlling the sympathetic output from these centers to the heart.

Part B included: No

Serial No. NHI-90

1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Elaboration of Catechol Amines by the Myocardium during Direct and Reflexly Induced Sympathetic Stimulation.

Principal Investigators: J. P. Gilmore, J. H. Siegel

Other Investigators: S. J. Sarnoff
Technicians: J. Miles , J. Cox, M. Edmunds

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.90
Professional: .70
Other: 1.20

Project Description:

Objectives - Methods Employed: To investigate the myocardial production and/or utilization of catechol amines as a result of direct and reflex stimulation of the cardiac sympathetic nerves. For this study cardiac output is controlled via an extra-corporeal perfusion pump and coronary blood flow, intracardiac and arterial pressures continuously monitored. In the early part of the study the method of Weil-Malherbe and Bone was used for the determination of catechol amines but, for the reasons discussed in the immediately preceding project report.* (the norepinephrine and epinephrine estimation in plasma study) the trihydroxyindole method as modified by Crout, was adopted. Experiments to date show:

1) Electrical stimulation of the stellate ganglia increases the concentration of catechol amines in coronary venous blood. This change can occur with no change in coronary arterial catechol amine concentration, mean arterial pressure, or stroke volume, and is observed when coronary flow is maintained constant.

2) The concentration of catechol amines in coronary venous blood is a direct function of both stimulus frequency and voltage.

*Norepinephrine and epinephrine estimation in plasma.

3) Continuous stimulation of the stellate ganglia can cause at least a partial depletion of myocardial catechol amines. This depletion appears to be able to be reversed by the I.V. infusion of norepinephrine.

4) Dichloroisoproterenol (DCI) blocks the hemodynamic responses to stellate ganglion stimulation but does not appear to significantly modify the myocardial release of catechol amines.

5) The changes in the coronary venous concentration of catechol amines during carotid occlusion are variable. However, it has been observed that when the coronary venous catechol amine concentration does increase during carotid occlusion, the increase is prevented by bilateral stellectomy indicating that during carotid occlusion the cardiac sympathetics are reflexly activated.

Proposed Course of Project: To determine whether the increase in arterial pressure which accompanies carotid occlusion modifies the myocardial utilization of catechol amines. Also, to utilize specific pharmacologic blocking agents in an attempt to separate the three factors which can contribute to the net amount of catechol amines found in coronary venous blood - utilization, production, and storage.

Part B included: No

Serial No. NHI-91
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Energetics of Myocardial Contraction Studied in the Dog Papillary Muscle - Active State and Inotropic Mechanisms of the Heart.

Principal Investigator: E. H. Sonnenblick

Other Investigator: Z. McCallum Technician: D. Edwards

Cooperating Units: None

Man Years (calendar year 1960)

Total:	1.13
Professional:	.83
Other:	.30

Project Description:

Objectives - Methods Employed: Although much work has been done to analyze the mechanics of skeletal muscle, relatively little definitive information has been obtained on the basis of which to differentiate the intrinsic factors involved in modifying the force of myocardial contraction. The present study was designed to allow a more precise analysis of myocardial mechanics as well as provide further insight into the mode of action of agents affecting myocardial contraction.

An apparatus was designed and built to study the mechanics of both the dog and cat papillary muscle. This apparatus allows for simultaneous measurement of changes in both initial and developed tension and length with sensitive control of temperature and chemical environment. Isotonic and isometric measurements are electively possible with concurrent calculation of force velocity relationships. A modified active state measurement has been developed which allows for instantaneous measurement of the intensity of the activity of the contractile elements of the muscle apart from inertial and viscous-elastic factors.

Major Findings: It was found that the contractile process can be dissected into three phases of active state - 1) the intensity of active state, determined from the instantaneous rate of developed tension following quick release, 2) duration of full intensity of the active state, determined by the ratio of rate of redeveloped isometric tension to initial rate of

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development of tension and 3) the decay of active state, determined by a measure of falling redeveloped tensions. These results reveal cardiac muscle to be different from skeletal muscle where only the duration of the active state has been shown to be variable; myocardium is shown to be able to vary its active state in both intensity and duration allowing for a variable rate of contraction as well as duration of contraction.

The active state measurements were studied with interventions which change the force of myocardial contractility - these studies provided insight into both the mode of action of these interventions as well as the underlying mechanisms by which the myocardium may alter the force of contraction. Increasing initial fiber length and increasing calcium produced an increase in the intensity of active state with no change in duration of full activity. Norepinephrine and increased rate increased intensity of active state while decreasing the duration of full activity. Decreasing temperature produced no change in active state intensity, while the duration of active state increased. Thus all these interventions produced an increase in developed tension. However, the mechanisms involved are not the same, but a resultant of at least these two intrinsic processes which may differ in extent as well as direction of change. The biochemical and thermodynamic implications of the findings are being explored.

Proposed Course of Project:
Work is proceeding to analyze the mode of action of K^+ in regard to Ca^{++} and norepinephrine. Data has been obtained to show that the mode of action of calcium and norepinephrine on the myocardium can be dissociated. Further, the mechanics of the myocardium are being explored in terms of force velocity curves. The intrinsic mechanisms of changing myocardial force of contraction are thus analyzed, and it is hoped that the underlying mechanisms will more readily be understood from this approach.

Part B included: No.

Serial No. NHI-92
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NHI
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Inotropic Mechanisms of the Intact Heart and their Regulation as Studied in an Innervated Isometric Dog Ventricle.

Principal Investigators: J. H. Siegel, E. H. Sonnenblick

Other Investigators: None

Cooperating Units: None

Man Years (calendar year, 1960)
Total: 1.19
Professional: .58
Other: .61

Project Description:

The ability of the intact heart to alter the peak tension which it can achieve as well as the rate of development of this tension from a given fiber length provides its ability to compensate for beat to beat changes in the resistance to its ejection.

The problem of differentiating the mechanisms responsible for these changes in contractility is complicated by the fact that in the presence of even the most minute ejection the heart fails, by virtue of this isotonic fraction, to achieve the peak tension of which it is capable during contraction. Consequently it has been difficult heretofore to quantitate changes in contractility on a beat to beat basis for the intact normally perfused and normally beating heart. Previous attempts to produce isometric contraction in the whole heart, while yielding valuable information, have suffered from limitations of compromised innervation, inaccurate volume measurements, and the difficulty of maintenance of a steady state.

A preparation has been designed in which the heart in situ has been totally by-passed by means of a pump oxygenator circuit. Aortic pressure and flow are maintained constant. Coronary flow is maintained in a normal fashion; the total coronary venous flow returning to the right side of the heart is prevented from entering the pulmonary circuit and is measured. Bronchial arterial flow is eliminated, and the small quantity of coronary venous flow returning to the left side of the heart is drained. An

undistended balloon is introduced into the cavity of the left ventricle, left ventricular volume increments are made by adding fluid into the balloon. Since the dynamic changes attendant upon left ventricular ejection are eliminated, it is possible for the heart to contract isovolumetrically and thus achieve peak tension with each beat.

Preliminary studies with this preparation have indicated that:

(1) At heart rates which allow adequate ventricular relaxation, direct cardiac sympathetic efferent nerve stimulation at any given ventricular volume and heart rate increases both the peak tension as well as the rate of development of this peak tension, over the control state, without change in the end diastolic pressure-volume relationships of the ventricle.

(2) Stimulation of the efferent vagus at a constant heart rate and ventricular volume appears to affect neither peak developed tension and rate of development of this peak tension nor to influence the end diastolic pressure volume relationships of the ventricle.

(3) While both the peak developed tension as well as the rate of development of this peak tension (at a given heart rate) vary directionally with end diastolic volume (and consequently fiber length) the ratio of the rate of development of tension to the peak developed tension appears to remain constant for any given state of contractility.

(4) Direct cardiac sympathetic efferent nerve stimulation (at a constant heart rate) causes the ratio of rate of development of peak tension to peak tension developed to increase. If frequency and voltage of the stimulus are maintained constant this relationship remains constant although at a higher level than control. Thereafter, the values for peak tension and rate of development of tension continue to vary directionally with end diastolic volume. It may eventuate that, by means of this relationship in the isovolumetric condition, a beat to beat index of contractility can be obtained.

(5) Increasing rate, per se, produces both an increase in the peak tension developed as well as an increase in the rate of development of this tension. Since the increment in tension developed is small compared to the increase in the rate at which it is developed there is an increase in the rate of developed tension-peak developed tension ratio as well. This is, however, small in magnitude compared to the increment brought about as a result of sympathetic efferent nerve stimulation at the same heart rate. However, the two effects appear to be additive with regard to their effect.

(6) Differences are observed between the manner in which calcium and catechol amines produce their increased contractility. A lower rate of developed tension-peak developed tension ratio obtains with calcium; the

duration of contraction is apparently only slightly changed with calcium but is much shorter after norepinephrine.

Proposed Course of Project: It is planned to extend these observations and also to compare the effects of isotonic and isometric contraction at the same volume level with regard to oxygen consumption.

Comparisons between the intact heart and an isolated papillary muscle strip are being made.

Part B included: No.

Serial No. NHI-93
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Functional Sympathectomy During Muscular Activity

Principal Investigators: J. P. Remensnyder, J. H. Mitchell

Other Investigators: S. J. Sarnoff
Technicians: C. Scott, F. Perry, D. Edwards

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.26
Professional: .65
Other: .61

Project Description:

Objectives - Methods Employed: This project represents a continuation of 1959 Project No. NHI-85. In addition to the previously described methods a fourth form of sympathetic stimulation was employed, namely, direct stimulation of the decentralized lumbar sympathetic chain. The effects of such an intervention were recorded in both exercising and resting extremities. In addition, a preparation was employed to study more exactly the pressure-flow characteristics of exercising and resting extremities during the interventions described. In brief, this employs a leg whose vessels are perfused by the arterial blood from a donor dog with necessary adjustments available for varying flow and pressures.

Major Findings: Two major findings are of importance: (1) the decrease in responsiveness to direct and indirect sympathetic stimulation on the vasculature of an exercising extremity; and (2) an increase in flow through an exercising extremity which results from employing centrally integrated sympathetic stimuli. These two phenomena have been studied systematically and data obtained in each instance correlate well over a wide range of oxygen consumptions.

That an exercising extremity demonstrates a decreased reactivity to sympathetic stimuli has important consequences for local metabolism described in the report Project NHI-84 (1959).

Proposed Course of Project: An attempt to elicit the mechanism of such a decreased reactivity, utilizing direct measurements of catechol amines across the extremities under various conditions.

Part B included: No.

Serial No. NHI-94
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: The Role of the Carotid Sinus in Maintaining the Relative Bio-Chemical Normalcy of Active Tissue.

Principal Investigators: J. H. Mitchell, J. P. Remensnyder

Other Investigators: S. J. Sarnoff
Technicians: C. Scott, F. Perry and D. Edwards

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.15
Professional: .65
Other: .50

Project Description:

Objectives - Methods Employed: This project represents an extension of 1959 Project NHI-84 and the methods employed are described in the report for Project NHI-85 (1959).

Major Findings: Have been partially described in report of 1959 Project No. NHI-84. In addition, certain other findings have accrued. A correlation between the peripheral resistance of an extremity and the extant oxygen consumption of that extremity has been noted. In addition a correlation exists between the degree of responsiveness to the various sympathetic stimuli employed and the extant oxygen consumption. It has been easily demonstrated that a linear relation exists between flow and oxygen consumption when the part is in at least partial oxygen debt.

The important consequence of these findings, is that by suddenly dropping carotid arterial pressure, one is able to demonstrate a substantial improvement in the local metabolism of an area undergoing augmented activity. The extension of this statement helps in clarifying the relation of the sympathetic nervous system and the local metabolism of the several parts of an organism when the latter vary their activity.

Proposed Course of Project: An attempt to correlate the augmented metabolism of a part with a decrease in catechol amine binding during centrally integrated sympathetic stimuli.

Part B included: No.

Serial No. NHI-95
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

PART A:

Project Title: Local Activation of the Kallikrein-Kallidin System During Electrically Induced Exercise.

Principal Investigators: J. P. Remensnyder, M. E. Webster

Other Investigators: None
Technician: W. Fisher

Cooperating Units: None

Man Years (calendar year 1960)
Total: .30
Professional: .20
Other: .10

Project Description:

Objectives - Methods Employed: As an extension of the suggestions of Fox and Hilton, the hypothesis was developed that in an area of sustained vasodilatation accompanying augmented activity (skeletal muscle exercise) the kallikrein-kallidin system is activated and exerts its vasodilating effect from the perivascular space. The substances would necessarily be removed from this space by the local lymphatics. With sustained vasodilatation accompanying activity, it should be possible to obtain measureable amounts of these substances from regional lymphatics.

The preparation requires the collection of lymph from the groin lymphatics of exercising and non-exercising legs. The issuing drops are immedately frozen until analysis is done. A bio-assay for activity of the enzyme is then employed utilizing a uterine strip from a rat in the follicular phase. In this procedure the lymph is added to partially purified human kallidinogen (prepared from human ACD plasma). The amount of kallidin formed is measured by its ability to contract the uterus.

Major Findings: Thus far, a measureable amount of the enzyme has been shown to be present in lymph issuing from an exercising leg. No detectable quantities of active polypeptide have been found. The results from control limbs have been equivocal. The lymph from most dogs contains a barely detectable level of enzyme as measured on the uterine strip, making the quantitation of the results difficult.

Proposed Course of Project: Investigation of alternate methods of measurement of the proteinase in resting and exercising limbs and correlation of the results with parameters of metabolic interest.

Part B included: No.

Serial No. NHI-96
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Studies on the Kallikrein System

Principal Investigators: M. E. Webster, J. V. Pierce, H. Moriya

Other Investigators: E. W. Emmart Technician: W. Fisher

Cooperating Units: H LCNP, A LPT

Man Years (calendar year 1960)

Total:	4-3/4
Professional:	2-3/4
Other:	2

Project Description:

Objectives - Methods Employed: The hypotensive proteinases called kallikreins exert their pharmacological action by enzymatic action on an α_2-2 globulin found in plasma called kallidinogen to release a polypeptide called kallidin. These pharmacologically active substances together with the various inhibitors involved have been the subject of further investigation.

Major Findings: Investigation of a number of synthetic esters for their ability to be digested by the kallikreins has revealed that the kallikreins derived from hog pancreas, human pancreas, human urine and human plasma hydrolyze those synthetic substrates which contain arginine as the specific amino acid residue. Arginine esters, benzoyl-L-argine amide and tyrosine methyl ester prevented the inhibition of human urinary and pancreatic kallikreins by human plasma. However, only arginine esters and the amide are effective in preventing the inhibition of human plasma kallikrein by diisopropylfluorophosphate. The major portion of the esterase activity in normal men's urine, as measured by its ability to digest p-toluenesulfonyl-L-arginine methyl ester, is due to urinary kallikrein. Acetone activates not only plasma kallikrein but other proteolytic enzymes which attack arginine esters.

Continued investigation of the possible identity of plasma kallikrein and the permeability factor of Niles and Wilhelm has shown that partially purified permeability factors contain plasma kallikrein and partially purified plasma kallikrein is active in producing an increase

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in permeability in guinea pigs. Correlation of the results obtained by both biological methods would suggest that they are measuring the same proteolytic activity. In cooperation with Dr. Ratnoff, investigation has found that human plasma kallikrein does not activate in Hageman deficient plasma. The data from these studies would suggest that the plasma of patients deficient in Hageman factor are not deficient in kallikreinogen, but rather lacking in kallikreinogenase or some prior enzymatic step required for the activation of plasma kallikrein. The question of the identity of the permeability factor, the glass-activated proteinase and plasma kallikrein cannot as yet be resolved. However, it is clear that Hageman Factor represents a common mechanism for their enzymatic release, but this does not exclude the possibility that the proteinases themselves may be different. Certainly, however, the simplest hypothesis would be that they were the same substance.

In preliminary studies on the development of rabbit antibody to human urinary kallikrein, considerable difficulty was encountered due to the lethality of the kallikrein preparations. Through purification a non-lethal, highly antigenic preparation has been obtained. Precipitin reactions by the agar gel diffusion technique, using this partially purified preparation, gave at least 3 precipitin bands indicating the heterogeneity of the antigen. Although the antisera diluted 1:300 times was found to inhibit the enzyme activity in dogs, immunochemical studies with more highly purified urinary kallikrein and in vitro esterolytic data suggest that a non-precipitating antibody had been formed. Since the antibody produced to human urinary kallikrein was also capable of inhibiting the vasodilator activity of pancreatic kallikrein, it would appear that these two proteinases are closely related chemically. However, crude human pancreatic kallikrein in agar gel diffusion plates gave at least two cross-reacting bands with the antibody. This suggests that some of the antigenic pancreatic components, other than that affecting vasodilator activity, may be excreted unaltered through the kidney or these data may merely reflect the presence in both urine and pancreas of similar or identical substances.

Major emphasis during the year has been placed on the purification of kallidin. Methods were developed for the purification of the polypeptide 4,000 times. When this purified material was chromatographed on carboxymethyl cellulose two widely separated polypeptides were obtained which had similar biological potency to that of pure bradykinin. Studies have continued on the purification of various human kallikreins. Methods have been found which will purify urinary kallikrein from 6 Frey units/mg to 100-200 Frey units/mg; pancreatic kallikrein from 4-8 units/mg to 40-60 units/mg; and plasma kallikrein from 0.3 units/mg to 9 units/mg.

Proposed Course of Project: Continued investigation of the components of the kallikrein system.

Part B included: No.

Serial No. NHI-97
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Action Potential Studies of Vagal Afferent Fibers in the Bull-frog (Rana Catesbeiana)

Principal Investigators: S. E. Downing and R. W. Torrance

Other Investigators: None Technician: T. Dale

Cooperating Units: None

Man Years (calendar year 1960):

Total:	1.08
Professional:	.75
Other:	.33

Project Description:

Objectives: Relatively few observations have been made on the afferent fiber population of the vagus nerve in submammalian forms. In the frog, action potentials have been recorded from pulmonary stretch fibers and from the pulmono-cutaneous artery. They have also been recorded from the carotid sinus nerve and from fibers originating in the atria. The purpose of this study was to investigate further the afferent population in the vagus nerve, in particular those fibers coming from the cardiovascular system.

Methods Employed: Pressures are recorded from the right atrium, the ventricle and the aorta simultaneously with the ECG. Single fiber preparations are obtained from slips of the peripheral vagus nerve and the electrical activity recorded using conventional electroneurographic techniques. Aortic pressure and ECG are recorded simultaneously with the action potentials utilizing a continuous motion camera. Pressures are altered by aortic occlusion or dextran infusion. Precise localization of receptors is determined by direct probing.

Major Findings: Cardiovascular afferent fibers were found coming from the atria, the ventricle, the conus arteriosus and the aortic arch. The frequency of discharge of the vascular fibers was related to arterial pressure and rate of change of pressure. The ventricular fibers appeared more closely related to the mechanics of ventricular contraction. Fibers were also recorded from the lungs which responded to stretch or inflation,

and from the pharynx, larynx, esophagus, trachea and stomach responding to direct probing.

Proposed Course of the Project: The project has been concluded.

Part B included: No.

Serial No. NHI-98
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: An Investigation of the Possibility that Slowly Adapting Pulmonary Stretch Receptors may Function as Chemoreceptors.

Principal Investigators: S. E. Downing

Other Investigators: None
Technician: T. Dale

Cooperating Units: None

Man Years (calendar year 1960):
Total: .58
Professional: .25
Other: .33

Project Description:

Objectives: The means by which the mammalian organism regulates its respiratory activity in relation to exercise is not completely understood. Deductive evidence suggests the presence of chemoreceptor tissue on the venous side of the circulation. None has been found, however, which have been demonstrated to be functional. The possibility that pulmonary stretch receptors of the Hering-Brucer type might function as chemoreceptors by alteration of response threshold was investigated.

Methods Employed: Electrical activity from single afferent fibers of slowly adapting pulmonary stretch receptors responding to inflation was recorded from the cervical vagus of the cat utilizing standard electroneurographic techniques. The interior chest wall was removed and the animal respired mechanically by a pump delivering a constant stroke volume. The animal was then respired with gases of high or low CO_2 content, high or low O_2 content and combinations thereof. The effects of altering blood pressure and cardiac output were investigated by opening and closing a snare on the inferior vena cava.

Major Findings: No alteration of the discharge frequency to lung volume ratio of the pulmonary inflation fibers was observed following marked changes of pO_2 and pCO_2 of the inspired gas. Marked reduction of blood pressure (and cardiac output) by occlusion of the

inferior vena cava also failed to alter the ratio. It was concluded that the threshold of response of the slowly adapting pulmonary stretch receptors responding to inflation is not altered by the pO_2 or pCO_2 of the blood or alveolar air under these experimental conditions.

Proposed Course of Project: The project has been concluded.

Part B included: No

Serial No. NHI-99
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Cardiovascular Response to Occlusion of Splanchnic Arteries.

Principal Investigators: J. H. Siegel, R. W. Torrance

Other Investigators: None Technician: T. Dale

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.08
Professional: .75
Other: .33

Project Description:

Objectives - Methods Employed: The possibility that the abdominal visceral circulation may contain sensory receptors which can influence vascular tone and myocardial function as do the baroreceptors of the carotid sinus and aortic arch has intrigued many investigators. Longcope examined the possible relationship of arteriosclerotic stenosis of the mesenteric vasculature, to systemic hypertension. He noted that of all of the subdiaphragmatic branches of the aorta, occlusion of the superior mesenteric artery alone produced a small but sustained elevation of mean arterial pressure in the dog. Subsequent work by Heymans, Bouckaert, Farber and Hsu, and Heymans, Bouckaert and Wierzuchowski, has indicated that in the dog non-thoracic baroreceptor sites probably exist and that arterial pressure alterations in the coeliac and mesenteric regions are probably responsible for local reflex distribution of blood in the abdominal viscera. However, these workers noted only minor alterations in systemic blood pressure with coeliac or mesenteric arterial occlusion even when the sino-aortic areas were denervated. Bennati and Chiggino have also demonstrated in the same species small increases in arterial blood pressure associated with portal venous hypertension after abdominal arterial occlusion.

Most recently, Sarnoff and Yamada have described substantial increases in arterial blood pressure and heart rate following coeliac and superior mesenteric or pancreatic arterial occlusion in the cat. It was suggested by these investigators that these effects might, at least in part, be due to a reflex whose afferent limb might be mediated

by the Pacinian corpuscular discharges. These discharges have been described by Gammon and Bronk as being synchronous with the arterial pulsations. However, Boyer and Scher attributed the changes of blood pressure in the cat to purely mechanical effects attendant on excluding a fraction of the vascular bed.

The splanchnic vascular bed supplied by the superior mesenteric and coeliac arteries receives approximately 25 per cent of the cardiac output and contains about an equal fraction of the total blood volume. Hence, occlusion of these vessels might in itself produce a significant increase in blood pressure both by increasing peripheral resistance and by infusing into the general circulation blood drained from vessels distal to the occlusion. In order to evaluate the significance and possible origins of the reflex effects described by Sarnoff and Yamada, an attempt has been made in this investigation to assess the relative importance of hydraulic and reflex effects of occlusion of the coeliac and superior mesenteric arteries.

Major Findings: The results of a variety of experiments which included completely isolating the splanchnic vascular bed, both with and without cross perfusion, and the recording of action potentials, indicate that both mechanical and reflex factors are operative. The occlusion of vessels per se increases the peripheral resistance. Also, blood runs off from vessels distal to the occlusion into the active circulation and so tends to increase heart output by acting as an infusion. Pressor reflex effects due to local irritation as ligatures were tightened on arteries were regularly found, but when these were excluded in cross perfusion experiments, reflex pressure changes could still sometimes be elicited by arresting the flow in splanchnic vessels.

It is suggested that chemoreceptors or ischemic pain receptors rather than baroreceptors are responsible for this response.

Proposed Course of Project: The results of this study have been submitted for publication.

Part B included: No

Serial No. NHI-100
1. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Myocardial Energetics and Oxygen Consumption in the Dog Papillary Muscle - Development of a Sensitive, Stable Micropolaragraphic Measurement of Oxygen.

Principal Investigators: E. H. Sonnenblick, Z. McCallum

Other Investigators: None Technician: D. Edwards

Cooperating Units: None

Man Years (calendar year, 1960)

Total:	1.13
Professional:	.83
Other:	.30

Project Description:

Objectives - Methods Employed: Although much information is available on factors affecting myocardial oxygen consumption in the intact organ, it is desirable to obtain more information concerning the relation of shortening to oxygen consumption under simpler geometric conditions. It has frequently been the practice to equate heat production with oxygen consumption in classical skeletal muscle studies; this assumption may well be invalid. According to classical views (Hill & Fenn), heat production is proportional to the heat evolved in the shortening process, above that developed with tension production, so that in a truly isometric contraction heat production actually falls. If the assumption that O_2 consumption follows heat production were true, O_2 consumption should fall in the contraction changing from a small amount of isotonic shortening to no isotonic shortening; - it is this assumption, which has only been tenuously supported by data from F. Fischer in 1936, on skeletal muscle that these studies were designed to challenge.

An oxygen polarograph has been designed and built with the aid of Mr. Peter Styles of St. Thomas Hospital, London, using a modified Tetronix 532 oscilloscope and micro-Clark electrodes covered with a teflon membrane. With this instrument, a micro-electrode, immersed in a solution containing oxygen, is subjected to a changing (saw-tooth) potential of 0 - 1.2 volts over a one second period, and corresponding changes in electric current recorded on a cathode ray oscilloscope, the output from the maxima auto-

matically gated and applied to a Sanborn D. C. coupling amplifier.

This instrument is to be used for recording changes in oxygen tension of a solution flowing past small strips of papillary muscle. Since the strip is quite small - (20 - 30 mg.) - the oxygen uptake will be correspondingly low; this will, in turn, necessitate a slow flow in order accurately to detect changes in the oxygen tension of the solution. A slow flow in turn requires that a small electrode will have to be used in order that the electrode O_2 requirements do not exceed availability. This means that the current output which is directly proportional to the cathode area will again be quite low, necessitating quite a large degree of amplification. As has been determined by manual current voltage curves, the higher the O_2, the shorter the plateau, and therefore the more likelihood of alinearity due to the residual current. In the high O_2 ranges, this could be a considerable handicap. With the present cathode ray polarograph, limits can be set for any concentration range by putting in two known gas concentrations and using them to set the limits; accordingly, any current produced below the wave of the lowest O_2 concentration can be completely ignored. The residual current problem may well be dissolved or diminished by this approach.

A satisfactory method for preparing the Clark type electrodes has been developed. A large size range of electrodes are presently being made and tested.

The advantages of this micropolarographic technique are:

(1) Using a generated saw-tooth voltage instead of amperometry and recording the entire current voltage wave form on an oscilloscope allows for immediate recognition of electrode poisoning or a change in membrane form. The danger of artefacts is thus minimized.

(2) Use of a saw-tooth, intermittent potential reduces electrode deposition, increasing stability.

(3) Sensitivity is extremely high and linearity is excellent.

(4) This instrument employs as its power supply, amplifier and monitor a Tetronix oscilloscope which has multiple other uses when not employed for the present experiments. Thus, the basic apparatus cost is much reduced while providing substantial advantages not now commercially available.

Proposed Course of the Project:

(1) The present micro-polarographic O_2 technique is being refined, further tested and applied to the study of O_2 consumption in the papillary muscle.

(2) The Fenn effect will be explored and related to O_2 consumption. The ability to measure and alter initial length and tension of a papillary

muscle has already been developed, and it is planned systematically to study the relative influence of tension development, shortening and rate on O_2 consumption. The effect of pharmacologic agents, such as norepinephrine and digitalis, on the efficiency of papillary muscle will probably also be studied.

Part B included: No.

Serial No. NHI-101

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: The molecular weight of myosin determined from light scattering measurements

Principal Investigators: William F. Harrington
S. Walter Englander
Martin Gellert, NIMH

Man Years (calendar year 1960):
Total: 1.5
Professional: 1.5

Project Description:

Objectives: Although it is now apparent that the determinations of the molecular weight of myosin utilizing the ultracentrifuge are all rather consistent and indicate a molecular weight of 600,000, published determinations of this parameter by the light-scattering technique appear to give significantly lower values. The present project was designed to examine some possible bases for this discrepancy with a view toward more securely establishing the molecular weight of this important protein.

Methods Employed: Light-scattering molecular weights are very sensitive to the refractive index increment for the protein. This property of myosin was determined by a new and improved technique coupled with careful measurement of concentration by dry weight determinations and spectrophotometry. Light-scattering measurements were made using standard techniques and apparatus.

Major Findings:

1. The true refractive index increment for myosin is considerably lower than previously reported and in good agreement with reliable values for other proteins.
2. The molecular weight determined is in much better agreement with the ultracentrifuge values.
3. Determination of the subunit molecular weight for myosin in concentrated guanidine hydrochloride is in good agreement with the ultracentrifuge values.

Significance to Heart Research: This work is aimed at a better understanding of the mechanism of muscular contraction.

Proposed Course of Project: Project terminated.

Part B included No X

Serial No. NHI-102
1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Biochemistry of muscular contraction

Principal Investigators: W. Wayne Kielley
Takamitsu Sekine

Other Investigators: Lisa M. Barnett (technical)

Man Years (calendar year 1960):
Total: 1 3/4
Professional: 1 1/4
Other: 1/2

Project Description:

Objectives: It was observed some time ago that the ATPase activity of myosin, the protein unit of the contractile mechanism, exhibits a biphasic response to titration of the SH groups, with a 3-4 fold stimulation of ATPase activity occurring when approximately 1/2 of the SH groups are titrated, further titration leading eventually to complete inhibition. The initial phase of this process is also characterized by a marked alteration in the substrate and activation specificities of the protein and during the course of the titration the ability of the molecule to react with another muscle protein, actin, (a property that is widely regarded as important in the mechanism of contraction) is lost. Identification of the amino acid composition of those portions of the molecule involved in these processes and their localization in the molecule should provide considerable insight into the biochemical process of muscular contraction.

Methods Employed: It was planned to approach the problem using differential labeling of the groups with radioactive SH reagents followed by tryptic digestion and application of the "fingerprint" technique to identify the cysteine containing peptides.

Major Findings: Treatment of myosin with radioactive N-ethylmaleimide sufficient to react with one of its sixteen sulfhydryl groups leads to a marked acceleration of its Ca^{++} activated hydrolysis of adenosine triphosphate and a complete loss of its ability to hydrolyze inosine or guanosine triphosphate. Furthermore, the hydrolysis of adenosinetriphosphate is no longer accelerated by ethylenediamine tetraacetate. This process is accompanied by the labeling of one of the cysteine containing peptides identified by the

"fingerprinting" procedure. In addition, it appears that this peptide contains not one but two cysteine residues and the titration of this second group occurs during the inactivation phase of the biphasic response to SH titration.

Significance to Heart Research: This work is aimed at a better understanding of the mechanism of muscular contraction.

Proposed Course of Project: Other details of the labeling sequence of myosin SH groups will be examined and attempts will be made to isolate and establish the structure of the peptide fragment referred to above.

Part B included No X

Serial No. NHI-103

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Structure - Function Relationships in Pancreatic Ribonuclease

Principal Investigators: C.B. Anfinsen
J.T. Potts

Man Years (calendar year 1960):
Total: 1
Professional: 1
Other: 0

Project Description:

Objective: To undertake studies aimed at further characterizing the active molecular structure in pancreatic ribonuclease essential for enzymatic activity.

Methods Employed: Purified, commercially available ribonuclease is further purified by a variety of column chromatographic techniques. The principal method of investigation currently involves attack by exopeptidases, carboxypeptidase and leucine amino peptidase, on a derivative of ribonuclease, correlating changes in structure as a result of enzymatic digestion with parallel alterations in activity and physical properties of the molecule. The derivative of ribonuclease employed is Ribonuclease S prepared, with modifications, by the technique of Frederic Richards. This involves digestion of ribonuclease by subtilisn at 7° C. resulting in a ribonuclease derivative with a single peptide bond split. This product is fully active but can be readily separated into fractions susceptible to extensive digestion by exopeptidases. Changes in activity on recombination of the fragments are then observed. The techniques employed include two-dimensional paper electrophoresis and chromatography, amino acid analyses on the Aminco Beckman automatic amino acid analyzer, and enzymatic analyses employing spectrophotometric methods.

Major Findings: To date, it has been found that the enzyme may be inactivated by the attack of carboxypeptidase on the C terminal end of the Ribonuclease S core or larger derivative fragment. The minimum requirements for retention of activity are being determined by kinetic studies and isolation of partly digested molecules.

Attack by carboxypeptidase on the smaller fragment also destroys activity when tested upon recombination.

The nature of the physical-chemical changes in the molecule resulting in these inactivations are being checked by ultraviolet spectrum shifts and other techniques.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

Proposed Course of Project: Further delineation of the changes produced by structural degradation of the ribonuclease molecule as approaches to the problem of the active configuration of this molecule, which is, in turn, an excellent model compound (as the first enzyme whose amino acid structure is completely known), for attempts at understanding the molecular operation of enzyme systems in general.

Part B included No X

Serial No. NHI-104
1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Structural studies on the myosin molecule

Principal Investigators: David Michael Young
W. Wayne Kielley
William F. Harrington, McCollum Pratt Institute

Cooperating Unit: McCollum Pratt Institute, Johns Hopkins University
Baltimore, Maryland

Project Description:

Objectives: It now appears established that the myosin molecule is a long rod-shaped molecule formed from three physically and chemically identical polypeptide chains, each coiled in the form of a helix; the three helices being wound together in the form of a rope. The present study was initiated in order to obtain further insight into the details of folding and association through investigation of the changes brought about by guanidine hydrochloride.

Methods Employed: Using the techniques of optical rotation, viscosity and ultracentrifugation as parameters, the action of a number of guanidine hydrochloride concentrations on native myosin has been studied.

Major Findings:

In the dissociation of myosin into its subunits, the first phase appears to be that of unfolding of the helices followed by a collapse of the three-stranded rod into a more globular shape with eventual dissociation of the three polypeptide chains.

The unfolding process as judged by optical rotation is perfectly reversible from any stage of the dissociation process.

Significance to Heart Research: There is no immediate significance to heart research. However, it is felt that a basic understanding of the myosin molecule must precede a unified concept of muscular contraction.

Proposed Course of Project: The conditions for refolding the myosin subunits and reassociating them will be examined in greater detail with a view to understanding the structure of the myofibril.

Part B included No X

Serial No. NHI-105

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The molecular structure of muscle proteins: homogeneity of the myosin subunits

Principal Investigators: W. Wayne Kielley
William F. Harrington
Parker A. Small

Other Investigator: Lisa M. Barnett (technical)

Man Years (calendar year 1960):
Total: 2 1/2
Professional: 2
Other: 1/2

Project Description:

Objectives: It is now well established that the contractile system of muscle is constructed, primarily, from the two proteins, myosin and actin. Recent developments have provided a rather detailed picture of the structure of the myofibril and the probable localization of these proteins in it. As a consequence, a new outlook has been emerging as to the mechanism of the process itself. At the same time, it has become apparent that our knowledge of the principal components has been too inexact to justify attempts to construct models of the structural elements and our information on the chemistry of their interaction too incomplete for any formulation of mechanism. As a first approach to these problems efforts were successfully directed toward more exact measurements of the molecular parameters of myosin, leading to the conclusion that this molecule has the form of a three-stranded rope. This, in turn, introduced the question of whether the three strands were chemically identical or not. The answer to this question is of fundamental importance to further elucidation of the fine structure of myosin and is the one toward which this project has been primarily directed during the past year.

Methods Employed: The electrochemical behavior of myosin subunits dissociated by concentrated urea has been examined by electrophoresis in a gel matrix. The peptide patterns (fingerprints) provided by two dimensional chromatography-electrophoresis of proteolytic digests of radioactively labeled myosin have been examined by radioautography.

Major Findings:

1. Myosin completely dissociated by urea migrates as a single component electrophoretically demonstrating that the myosin subunits are electrochemically homogeneous.

2. Modifications of the "fingerprinting" technique have now clearly demonstrated that the number of radioactive peptides obtained by tryptic digestion of radioactively labeled myosin are compatible only with the proposition that the subunits of myosin have the same amino acid sequence. With these two pieces of evidence, it now appears conclusive that the myosin molecule is a trimer of a single chemically and physically homogeneous polypeptide unit.

Significance to Heart Research: This work is aimed at a better understanding of the mechanism of muscular contraction.

Proposed Course of Project: Although myosin appears to be a three-stranded rope structure, it is apparent that one portion of the molecule presents some more complex folded structure. This portion of the molecule possesses the biochemically active sites and its localization along the long rod-like myosin molecule is important in understanding the packing and behavior pattern of the molecules in the cellular structure from which they have been obtained. Present approaches to this problem are: 1) investigation of the unfolding process brought about by guanidine hydrochloride, and 2) identification of the two ends of the polypeptide chains (subunits) and localization of these in the large molecular fragments resulting from very brief proteolytic digestion of myosin.

Part B included Yes X

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Kielley, W.W., and W. F. Harrington, A model for the myosin molecule, Biochim. Biophys. Acta, 41, 401 (1960).

Small, P.A., W. F. Harrington and W.W. Kielley, The electrophoretic homogeneity of the myosin subunits, Biochim. Biophys. Acta, in press.

Serial No. NHI-106

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A chemical study of amino acid lipid complexes obtained from tissue

Principal Investigators: Richard W. Hendler
Evan C. Horning, LCNP, NHI
Majorie G. Horning [redacted]

Cooperating Unit: Laboratory of Chemistry of Natural Products, NHI

Man Years (calendar year 1960):
Total: None

Project Description:

Objectives: To isolate and chemically characterize the amino acid lipid complexes obtained from tissues.

Methods Employed and Major Findings: Since the last report, attention has been shifted to the metabolic aspects of the lipid-amino acid complexes. More effort will be spent on establishing whether or not all or particular complexes are involved in metabolic sequences. (See reports NHI-107 and NHI 108). This decision is not based on a decreased evaluation of the purely chemical approach but rather on the limitation of time available. It is hoped to shift back to the chemical characterization as early as possible.

Significance to Heart Research: The possible relation of lipid metabolism to the process of protein synthesis is being considered. Lipid metabolism is currently believed to exert a substantial influence in degenerative changes of the heart and blood vessels. If these amino acid lipid complexes do play an important role in the metabolism of tissues in general and the circulatory system in particular, a knowledge of their complete structure will be very important.

Proposed Course of Project: See "Methods Employed" above.

Part B included No X

Serial No. NHI-107

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Investigation of the ribonucleoprotein granules of rat liver ergastoplasm as sites for specific synthesis of cytoplasmic proteins

Principal Investigators: Richard W. Hendler
Elbert A. Peterson, NCI
Edward L. Kuff, NCI

Cooperating Unit: National Cancer Institute

Man Years (calendar year 1960):
Total: See report of NCI, NCI-365

Project Description:

Objectives: Current ideas in protein synthesis fix the ergastoplasm as the major cellular site for protein synthesis. The ergastoplasm consists of a lipid membrane, studded with ribonucleoprotein granules approximately 150 Å in diameter. It is believed that information for making particular proteins is contained in the configuration of the nucleic acids of these granules. If this is so, one should expect that the granules are heterogeneous. We are trying to fractionate these granules and determine if different granules do make different cytoplasmic proteins.

Methods Employed and Major Findings: Drs. Kuff and Peterson have been trying to improve the procedure for releasing the ribonucleoprotein granules from the ergastoplasmic membrane and the subsequent chromatography of the particles. Some success in this direction has been achieved and these activities are described in the report of the National Cancer Institute, NCI-365.

Significance to Heart Research: The dry weight of the body and its soft tissue structural material is mainly protein. The Heart Institute is concerned with understanding the normal function of heart tissue. The heart as a muscle is in a constant state of protein synthesis and degradation. Biochemical knowledge is based on the thesis that basic biochemical mechanisms are similar in the varied types of living tissue. The ability of a cell to function normally is based on the direction it receives. This direction must be in the form of chemical structures. It seems likely that many defects in

tissues may be traced to defects in the processes determining aspects of specificity in the cell's metabolism.

Proposed Course of Project: Drs. Peterson and Kuff will try to improve the separation of granules already obtained by employing a more gradual elution gradient.

I shall then investigate, with Kuff, the best manner of releasing newly synthesized soluble protein from in vivo labeled ribonucleoprotein granules. We three will investigate the metabolic activity of various fractions of liver with respect to amino acid uptake and turnover. Finally, we hope to use all accumulated information in an attempt to see if different classes of ribonucleoprotein granule, labeled in vivo, will release different specific soluble proteins characteristic of rat liver.

Part B included: No X

Serial No. NHI-108

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A study of the mechanism of protein biosynthesis in the hen oviduct

Principal Investigator: Richard W. Hendler

Other Investigator: Ethel Love (technical)

Man Years (calendar year 1960):
Total: 1.25
Professional: 0.75
Other: 0.50

Project Description:

Objectives: The mechanism of protein biosynthesis is one of the most important areas in biochemistry about which the least is known. It is the purpose of this work to study the basic reactions of protein biosynthesis in a tissue highly specialized for performing this function, the oviduct of the laying hen.

Methods Employed and Major Findings: In connection with the main current of this project, several ancillary problems had to be pursued.

1. Synthesis of high specific radioactivity amino acids of the natural variety. 20 ml of $BaC^{14}O_3$ were used as the sole carbon source for growing algae. 45% of the starting radioactivity was obtained in the final isolated protein. The other radioactive components were distributed to NIH investigators.

2. Investigation of the sensitivity of the X-ray film autographic technique for detecting C^{14} and H^3 on paper chromatograms.

The exact density of darkening of X-ray film was studied as a function of level of radioactivity, time of exposure, type of X-ray film used, effects of scintillation phosphors added to thepaper, and attempts to impregnate the paper with silver salts. The method has been standardized and the most effective X-ray film selected. Additions of all phosphors was without affect and the silver impregnation technique could not be effectively accomplished because of technical limitations.

3. A new technique has been developed for distinguishing free amino acid, carboxyl-bound amino acid and carboxyl-bound fatty acid in lipid materials. It is based on chromatography of hydroxamic acid derivatives on a Fe^{+++} carboxyl adsorbent.

Main Problem - It was found that specific sites exist in the lipids which can distinguish L-valine from D-valine or L-serine. Evidence was obtained which points to a covalent link between the carboxyl group of the amino acid and a lipid component. Preliminary findings suggest the existence of a lipo-peptide thought to be involved in the protein synthetic process of the oviduct.

Significance to Heart Research: The dry weight of the body and its soft tissue structural material is mainly protein. The Heart Institute is concerned with understanding the normal function of heart tissue. The heart as a muscle is in a constant state of protein synthesis and degradation. Biochemical knowledge is based on the thesis that basic biochemical mechanisms are similar in the varied types of living tissue. By studying a tissue highly specialized with respect to the process of protein synthesis (oviduct of the laying hen) it is hoped that knowledge of this vital phenomenon will be applicable to the tissues of man.

Proposed Course of Project: The isolation, purification and characterization of the lipid-amino acid complexes will be pursued. The possible role of these compounds in protein biosynthesis will be further studied.

Part B included: No X

Serial No. NHI-109

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A study of the possible role of amino acid-lipid complexes in the metabolism of E. coli

Principal Investigators: Richard W. Hendler
Richard M. Roberts, Carnegie Institute of Washington
Patrick F. Duggan

Cooperating Unit: Carnegie Institute of Washington, Washington, D.C.

Man Years (calendar year 1960):
Total: 1/3

Project Description:

Objectives: The possible role of lipids in the biosynthesis of proteins of the hen oviduct has been indicated by work with this tissue. A similar study in a completely different tissue capable of large scale protein synthesis was deemed desirable in order to study the generality of the indicated findings with the hen oviduct. Furthermore, a role of the amino acid lipid complexes in amino acid transport is indicated by our recent studies with E. coli. Therefore, our objectives now include a study of the transport process. The Carnegie group is considered to be the leading experts in the field of E. coli metabolism and a collaboration of this kind appears to be very suitable for the problem under study.

Methods Employed and Major Findings: A thymine, arginine, uracil requiring mutant of E. coli was incubated for 12 seconds with a mixture of radioactive amino acids and this was followed by addition of unlabeled amino acids and subsequent incubation of 120 seconds. The cells were broken and all fractions examined for uptake and loss of radioactivity and chromatographic behavior of lipid-contained radioactivity. It was found that the cell wall and ribosomes of the 70s and 30s classes were rapid in their uptake of radioactivity, but not the soluble fraction. Although the ribosomes contain only a small fraction of lipid compared to cell wall, they yielded twice the radioactivity in a lipid extract. Chromatographically, the radioactive lipid material from the ribosomes was the same as that obtained from the cell wall material. Therefore, the active protein synthesizing granules may be physically associated with the cell wall membrane in the intact cell.

Significance to Heart Research: The possible relation of lipid metabolism to the process of protein synthesis is being considered. Lipid metabolism is currently believed to exert a substantial influence in degenerative changes of the heart and blood vessels. A general association of lipid and protein metabolism can be established only by studying several different tissues.

Proposed Course of Project:

1. To further characterize amino acid-lipid complexes from structural fragments of different strains of E. coli.
2. To examine mutants of E. coli, unable to concentrate a particular amino acid for ability of the mutant to make an amino acid-lipid complex with that amino acid.
3. To study in general the possible involvement of amino acid-lipid complexes in the processes of amino acid transport and protein synthesis in E. coli.

Part B included: No X

Serial No. NHI-110

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Investigation of structural and functional relationships in ribonuclease

Principal Investigator: Frederick H. White, Jr.

Other Investigators: None

Man Years (calendar year 1960):
Total: 1
Professional: 1
Other: None

Project Description:

Objective: To investigate the structural and functional relationships of ribonuclease and other enzymes for the purpose of obtaining more information on the mechanism of enzyme action.

Methods Employed: Methods that were developed in this laboratory have been used for the reduction and reoxidation of the disulfide bonds of ribonuclease, and for the alkylation of reduced ribonuclease. Ribonuclease and its derivatives have been purified by chromatography on carboxymethyl cellulose or XE-64. In comparative studies of native, reduced, and reduced-reoxidized ribonucleases, optical rotatory dispersions, viscosities and ultraviolet spectra have been obtained. In a search for differences in the locations of disulfide bonds in native and reoxidized ribonucleases, proteolytic methods were used for the degradation of these proteins, followed by chromatographic and electrophoretic techniques for separating the resulting peptides into characteristic patterns. Methods have been elaborated for the "thiolation" of ribonuclease, i.e., reaction with N-acetyl homocysteine thiolactone, by which sulfhydryl groups are introduced into the ribonuclease molecule. Various methods of assay have been employed to determine the effects of thiolation on the enzymatic activity of ribonuclease. Complete acid hydrolysis of thiolated-alkylated or reduced-alkylated ribonuclease, followed by analysis for amino acid content either by the Spinco model MS amino acid analyzer or by various other methods of chromatography, has been employed to determine the extent of thiolation or of reduction. Titration with p-chloromercuribenzoate has been used to determine the number of sulfhydryl groups produced in the protein by thiolation or reduction. As an additional method of measuring the extents of thiolation and

reduction, the protein was alkylated with C^{14}-labeled iodoacetate, and the extent of radioactive labeling was determined.

Major Findings:

1. Comparative studies of native, reduced, reduced-alkylated, and reduced-reoxidized ribonucleases were brought to completion. The results of this investigation strongly indicate that native and reduced-reoxidized ribonucleases possess the same secondary and tertiary structures. This conclusion is of theoretical significance with regard to the mechanism of protein biosynthesis, suggesting that the formation of secondary and tertiary structures may be governed by information contained in the amino acid sequence rather than by the template from which the sequence is determined.

2. Reduction and reoxidation of protein disulfide bonds has been extended to lysozyme and insulin. The former has been fully reduced and then reoxidized with a regeneration of enzymatic activity comparable to that observed for ribonuclease, treated in the same manner. Reduced insulin, however, could not be made to regenerate hormonal activity on reoxidation. The latter finding suggests that the spontaneous formation of secondary and tertiary structures, observed for ribonuclease and lysozyme, occurs only when the native protein consists of a single protein chain.

3. A kinetic study of the reoxidation of reduced ribonuclease has revealed a lag period of about three hours in the appearance of enzymatic activity, while the entire reoxidation time is 20 hours. Additional experiments performed in collaboration with Drs. C. B. Anfinsen, M. Sela, and E. Haber indicate that the regeneration of native rotatory and spectral properties, as well as the disappearance of sulfhydryl groups, are relatively rapid during the reoxidation period.

4. Thiolation of ribonuclease followed by alkylation with iodoacetate results in an apparent maximum of two S-carboxymethyl homocysteinyl groups substituted into the protein molecule. Thiolation results in a transient rise in the specific activity toward ribonucleic acid above that of native ribonuclease and a marked decrease in activity toward uridine and cytidine-2',3'-cyclic phosphates. Chromatography of partially thiolated ribonuclease on carboxymethylcellulose has revealed several intermediates.

5. Thiolation of lysozyme produced no apparent change in its specific activity.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical nature with obvious bearings on tissue metabolism in general.

Proposed Course of Project: Studies on reduction and reoxidation of disulfide bonds will be extended to other proteins. Reduction and reoxidation of chemically modified ribonucleases will be tried in an effort to obtain more information regarding the features of amino acid sequence that govern the formation of native secondary and tertiary structures. Investigation of the effects of thiolation on ribonuclease enzymatic activity will be continued, and efforts will be made to determine the locations of the substituent S-carboxymethyl homocysteinyl groups in order to gain further insight into the relationship between molecular structure and enzymatic function.

Part B included Yes X

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

White, F.H., Jr., Regeneration of native secondary and tertiary structures by air-oxidation of reduced ribonuclease, J. Biol. Chem., in press.

Serial No. NHI-111

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on heparin and lipoprotein lipase

Principal Investigator: Edward D. Korn

Other Investigators: Bertha Neal (technical)

Man Years (calendar year 1960):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: To study the structure and metabolism of heparin and related mucopolysaccharides, the relation of heparin to lipoprotein lipase and the role of lipoprotein lipase in fat metabolism.

Methods Employed and Major Findings: Olive oil, corn oil, cream and cocoa butter were fed to rats whose thoracic ducts had been cannulated. The chyle was collected and chylomicrons prepared from it. These chylomicrons were then incubated with pancreatic lipase and lipoprotein lipase. The free fatty acids that were liberated by enzymic hydrolysis were isolated and the molar percentage composition determined by gas-liquid chromatography and compared to that of the substrate triglyceride. Also, the relative amounts of free fatty acid and glycerol produced during hydrolysis were determined. The results show clearly that whereas pancreatic lipase hydrolyzes preferentially fatty acids esterified at the α-position of a triglyceride, lipoprotein lipase hydrolyzes α- and β-esters equally well. Nor does lypoprotein lipase have a specificity among the long chain fatty acids, saturated and unsaturated.

Significance to Heart Research: Heparin is not only a potent anticoagulant but also appears to be involved in lipoprotein metabolism. Lipoprotein lipase is an enzyme which probably plays a major role in lipid transport and metabolism.

Proposed Course of Project: Efforts to purify lipoprotein lipase will be continued. A study has been begun on the effects of polyanions and polycations on the activity of lipoprotein lipase.

Part B included Yes X

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Linker, A., Hoffman, P., Meyer, K., Sampson, P. and Korn, E.D., The formation of unsaturated disaccharides from mucopolysaccharides and their cleavage to α-keto acid by bacterial enzymes, J. Biol. Chem. 235, 3061-3065, 1960.

Serial No. NHI-112

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of the structure of lysozyme

Principal Investigator: Robert E. Canfield

Other Investigators: Arthur Martin (technical)

Man Years (calendar year 1960):
Total: 1
Professional: 1
Other: None

Project Description:

Objective: To determine the amino acid sequence of the enzyme, lysozyme, and to initiate studies relating this enzyme's structure to its biochemical function.

Methods Employed: During the past year a technique has been perfected in this laboratory which permits the separation of the component peptides of lysozyme following reduction and alkylation of sulfhydryl groups and trypsin digestion of the protein. This technique consists of a gradient elution from phosphorylated cellulose ion exchange columns using pH and salt gradients of a volatile buffer, ammonium acetate. The amino acid analyses of the peptides obtained by this method provide the background information and material necessary for further sequence work. Current attempts at specific blocking of selected sites during trypsin digestion are aimed at providing information which will allow the peptides to be assembled in their proper order.

Significance to Heart Research: This work has provided a technique which should facilitate attempts to obtain protein sequence information. It is part of an effort by this laboratory to correlate protein structure information with enzyme function as well as to obtain basic information regarding protein biosynthesis and genetic control of protein structure.

Proposed Course of Project: Once the peptide amino acid analyses are complete, information concerning their "internal" sequences will be obtained by digestion with enzymes other than trypsin and by specific chemical methods. It is hoped that chemical modification at certain specific sites in the reduced alkylated enzyme will lead to selective trypsin cleavage and thus provide an opportunity to arrange the entire chain of approximately 130 amino acids in its

proper linear sequence. It is also planned to study the differential incorporation of a "pulse" of C^{14} labelled arginine into the eleven arginine positions of lysozyme at the time of synthesis by hen oviduct. It is hoped that this information will provide definite evidence concerning the direction of synthesis of the polypeptide chain at the site of formation.

Part B included No X

Serial No. NHI-113

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of the mechanism of genetic control of protein synthesis

Principal Investigators: William J. Dreyer
Elward Bynum
Thomas Merigan

Other Investigators: John Bishop (Visiting Scientist, July-August)

Cooperating Units: George Streisinger (collaborator)
Institute of Molecular Biology
University of Oregon

Man Years (calendar year 1960):
Total: 2 1/2
Professional: 1 1/2
Technical: 1

Project Description:

Objectives: Recent genetic studies have made it clear that the genetic material, deoxyribonucleic acids (DNA), of living organisms exerts rigid control over the synthesis of proteins. It appears that a change in the chemical structure of DNA due to a mutation can result in a change in the structure of a protein synthesized under the control of the altered "gene." The structural analysis of such altered proteins should provide useful data relating to the translation of genetic information into protein structure. Ultimately, the knowledge gained should also be of considerable value in the study of structure and function of proteins and enzymes.

The genetic systems and the proteins currently being studied are those of the very simple "organisms," bacterial viruses.

Major Findings: A. Bacteriophage lysozyme structure: Sequence studies are in progress. A great deal of information has been collected using some of the new methods discussed below. This project is being actively pursued since the information to be gained is of vital importance to the over-all program dealing with genetic coding problems.

B. Development of methods for the study of protein structure: Each advance in methodology seems to permit an improvement in both the quantity and quality of work that can be done on projects requiring protein structure studies. For this reason we have continued to direct some of our efforts toward this end. Several useful methods have been developed in the past year.

1. One particularly useful method permits us to separate all of the usual amino acids in one dimension by means of high voltage electrophoresis. A dozen or more analyses can be carried out in a few hours. While the method is primarily a qualitative one at present, we have found the quantitative results to be more than adequate for many purposes.

2. Theoretically, at least, the determination of the sequence in which a large number of peptides derived from a protein were originally arranged in that protein should become an extremely simple problem if one could produce exactly the "overlaps" needed without relying on chance. This end might be accomplished by using the proteolytic enzyme, trypsin. This enzyme hydrolyzes proteins at both lysine and arginine residues. A simple method has been worked out permitting us to block all lysine residues. Trypsin then splits only the arginine bonds. The blocked lysines can then be freed by simply lowering the pH. This approach yields lysine overlaps with relative ease.

Experiments designed to block arginine residues easily and in a reversible manner have not been as successful. However, it seems possible that one of several approaches tested in a preliminary way may yet prove satisfactory.

3. Another minor but useful method has been worked out which permits us to prepare the dinitrophenyl derivative of peptides directly on the paper used for separation. Elution and appropriate analysis then permits rapid identification of amino terminal groups of peptides.

C. Genetic aspects of the problem: During the past year, work (pursued in collaboration with Dr. George Streisinger of the University of Oregon) on the genetic aspects of the bacteriophage lysozyme system has progressed rather well. A selection system has been devised which permits the isolation of a large number of mutants, each of which produces an altered lysozyme. Fine structure genetic mapping is possible using various techniques developed. A second type of selection system has resulted in the isolation of mutants which appear to produce inactive enzymes. This class of mutants may lead to certain insights into the nature of enzyme "active centers".

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. This work, in general, is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

Proposed Course of Project: Experiments are continuing in an effort to gain information on the "genetic code" and the role it plays in controlling protein structure. Since the bacterial virus lysozyme continues to provide a most promising experimental system for these studies, work on the structure of this enzyme is being carried out. During the course of this work, further

effort will be made to devise new, simpler, and more powerful methods for the study of protein structure. This aspect of the problem continues to be rate limiting.

The selection for interesting enzyme mutants will be continued. The fine structure genetic map will be extended. Ultimately, it is by the determination of the nature of the changes produced in the enzyme as a result of mutation (particularly when induced by chemical mutagens with known mechanisms of action) that we hope to begin to gain an insight into the nature of the "genetic code."

Part B included Yes X

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Dreyer, W.J., An experimental approach to genetic coding problems. Conf. on Molecular and Radiobiology, in press.

Dreyer, W.J., Genetic control of bacteriophage lysozyme structure and some genetic coding problems. Structure and Function of Protein, Brookhaven Symposia in Biology, No. 13 (1960), in press.

Serial No. NHI-114

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies with respect to reduction and oxidation of ribonuclease

Principal Investigators: Edgar Haber
C. B. Anfinsen

Other Investigators: None

Man Years (calendar year 1960):
Total:1 1/2
Professional: 1 1/2

Project description:

Objectives: To gain information on the relationship between the nature of the folded structure of ribonuclease and its enzymatic activity.

Methods Employed:
1. Reduction of ribonuclease with chemical reducing agents with subsequent separation of various stages of reduction by column chromatography. These are subsequently studied with respect to structure and enzymatic activity.
2. Electrolytic reduction of ribonuclease and studies of products of reduction, as outlined above.
3. Oxidation of fully reduced ribonuclease and derivative materials in the presence of various substances with subsequent study of chemical, physical and enzymatic properties of the products.

Major Findings:
1. Reduction of ribonuclease by sulfhydryl reducing agents results in two products, fully reduced ribonuclease and material in which three of the four disulfide bridges are cleaved. Both these materials are enzymatically inactive.
2. Electrolytic reduction may specifically and sequentially cleave the disulfide bridges of ribonuclease. The product with a single bridge cleaved appears to be enzymatically inactive.
3. a. Oxidation of reduced ribonuclease in buffered aqueous solutions at neutrality has previously been shown to yield a product which is indistinguishable from the native molecule in structure and enzymatic activity. However in the presence of urea, guanidine or compounds related to tyrosine, a structured, but enzymatically inactive material is at least in part related to

hydrogen bonding, perhaps of the tyrosine residues.

3. b. The 104 amino acid derivatives of ribonuclease known as S protein may be fully reduced and then oxidized to form a product which when combined with the 20 amino acid peptide known as S peptide results in full enzymatic activity. (See 1960 Project Report by C. B. Anfinsen and John Potts)

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearing on tissue metabolism in general.

Proposed Course of Project: The nature of the reduced and oxidized products of ribonuclease will be investigated further in order to elucidate the requirements of tertiary structure necessary for enzymatic activity as well as the nature of the code influencing correct folding of the reduced molecule.

Part B included Yes X

Serial No. NHI-114

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Haber, Edgar and C. B. Anfinsen, Regeneration of enzyme activity by air oxidation of reduced, subtilisin modified ribonuclease, J. Biol. Chem., in press.

Haber, Edgar, Specific reduction of one of the disulfide bonds in ribonuclease by controlled potential electrolysis, J. Biol. Chem., in press.

Serial No. NHI-115

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the metabolism of triglycerides by adipose tissue and liver (1959 Serial No. NHI-102)

Principal Investigator: Martin Rodbell

Man Years (calendar year 1960):
Total: 3/4

Project Description:

Dr. Rodbell left in August for a year in the Laboratory of Professor Brachet in Brussels, and consequently details of his project are not submitted at the present time. The work is, however, covered in the report by Drs. Scow and Chernick of the NIAMD, with whom Dr. Rodbell collaborated during the past year.

Serial No. NHI-116
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: I Biochemistry of the Differentiating Slime Mold. II. Mechanism of Induced Enzyme Formation.

Principle Investigator: Barbara E. Wright

Technical: G. Liddel
G. McNeil
K. Chaney

Man Years (1960)
Total:
Professional:
Other:

Project Description:

Objective: (I) To analyze the biochemical events occuring during development and to correlate these with the morphological changes. (II) to determine if an inducible protein (β galactosidase) is synthesized at the same rate before as after induction.

Major Finding:

I. (a) (with B. Bloom) 6 C^{14} glucose and 1-C^{14} glucose were incubated with slime molds at 5 stages of development and the radiochemical yields of $C^{14}O_2$ determined. The ratio of $6CO_2$ to $1CO_2$ was 0.7 in the amoebae, 1.3 at the pseudoplasmodium and preculmination stages, and 0.3 at the fruit stage. Three possible pathways to account for a ratio greater than 1.0 were dismissed because the specific reactions could not be detected in slime mold extracts.

A determination of the specific radioactivity of intracellular glucose (following exposure of the cells to C^{14}-glucose) demonstrated that permeability of exogenous glucose at the amoebae stage was only of the order of one hundredth as good as at preculmination. Taking this into consideration, the relative radiochemical yields of $C^{14}O_2$ during development decreased by an order of magnitude. This was interpreted as reflecting an increased dilution of the products of C^{14} glucose metabolism by similar unlabeled compounds of endogenous origin.

Part A cont'd.

I(b) (with G. Liddel)

Respiration of the slime mold during differention, based on dry weight and cell number, was determined at various stages in the presence and absence of glucose. The endogenous Q_{O_2} was maximal at the amoebae stage, dropped to a plateau between the pseudoplasmodium and preculmination stages, and then dropped again at the fruit stage. Glucose stimulated respiration maximally at the fruit stage, raising the Q_{O_2} to the values obtained in the plateau region in the absence of glucose. Various indirect lines of evidence suggested that a critical depletion of endogenous substrates occurs at the culmination stage of differentiation.

I(c)

A heat-labile proteolytic-type agent(s) present only in extracts prepared at the amoebae stage of development was discovered to specifically destroy isocitric-and G6P-dehydrogenases. These enzymes could be fully protected by the presence of their substrates. Glutamic acid dehydrogenase and glutamic-pyruvic transaminase were not attacked. Knowledge of this differential enzyme destruction as a function of the stage of differentiation has, in conjunction with related studies, suggested the possibility that enzyme activities in vivo may be controlled by sequential endogenous substrate utilization (and inhibition), thus accounting for certain metabolic changes occuring during differentiation.

II (with G.McNeil)

Under condition of logerithmic growth and maximal induction with melibiose, (a powerful inducer of β galactosidase), a 30 second pulse of highly labeled leucine is given to a culture of E. coli. The enzyme is purified and its specific radioactivity compared to the average cellular protein. The enzyme was slightly colder. By comparing this result with a comparable one in (progress) in which no inducer or a poor inducer is used, we will be able to tell if thereis a change in the rate of synthesis of this specific protein during induction.

Proposed Course of Project:

I. The next investigation will deal with the in vivo turnover of glutamic acid as a function of the stage of slime mold differentiation. II. The experiments on β-galactosidase may be repeated with a recently obtained strain having a very high "basal level" of enzyme.

Part B Included YES

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Publications:

1. Heftman, E., Wright, B.E., and Liddel, G.U. "Identification of a sterol with acrasin activity in a slime mold". J.A.C.S. 81 6525 (1959).

2. Wright, B.E., "On Enzyme-Substrate Relationships During Biochemical Differentiation", Proc. Nat. Acad. Sci. 46 798 (1960).

3. Wright, B.E., and Anderson, M.L. "Protein and Amino Acid Turnover During Differentiation in the Slime Mold. I Utilization of Endogenous amino acids and protein", Biochim. et Biophys. Acta 43 62 (1960).

4. Wright, B.E., and Anderson, M.L., "Protein and Amino Acid Turnover During Differentiation in The Slime Mold II Incorporation of S^{35} methionine into the amino acid pool and into protein" Biochim et Biophys. Acta 43 67 (1960).

5. Wright, B.E. and Bloom, B. "In vivo investigations of glucose catabolism in a differentiating slime mold" Bact. Proc. 1960.

6. Wright, B. and Bloom, B. " In vivo evidence for metabolic shifts in The Differentiating Slime Mold". Biochim et Biophys. Acta (in press) 1960.

7. Heftman, E., Wright, B.E., and Liddel, G.U., "The Isolation of Δ^{22}-stigmasten-3β-ol from Dictyostelium discoideum". Archives Biochem. and Biophys. (in press) 1960.

8. Liddel, G.U. and Wright, B.E., The Effect of Glucose on Respiration of The Differentiating Slime Mold, Developmental Biology, 1960, in press.

Serial No. NHI-117
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Lactate Fermentation by Butyribacterium rettgeri.

Principle Investigator: Charles L. Wittenberger

Professional: Martin Flavin

Technical: K. Chaney
G. Hunt
G. Henderson

Man Years (1960)
Total:
Professional:
Other:

Project Description:

Objectives: Butyribacterium rettgeri carries out a modified butyric acid type fermentation of lactate, the major products being butyric acid, acetic acid and carbon dioxide. Barker and coworkers have reported that this organism requires lipoic acid for growth when lactate serves as the carbon source but does not require lipoic acid when pyruvate or glucose is substituted for lactate in the growth medium. These results suggested that lipoic acid was not involved in the classical α-keto acid oxidation sequences but rather was functioning in a new and perhaps unique metabolic capacity. Studies by Barker's group have also revealed that the acetate formed by this organism is partially derived from the condensation of two moles of carbon dioxide.

This investigation is directed toward resolving the role of lipoic acid in lactate dissimilation by B. rettgeri and toward determining the pathway of acetate formation from carbon dioxide. It is hoped that information derived from this study will not only provide a basis for a better understanding of the metabolism of the organism but might also contribute to the general knowledge of the metabolism of one carbon units.

Part A cont'd.

Major Findings:

Preliminary studies have indicated that pyruvate is an intermediate in the formation of acetate and butyrate from lactate. Lipoic acid appears to be involved in the conversion of lactate to pyruvate but not in the subsequent dissimilation of pyruvate.

When B. rettgeri cells are washed extensively, their ability to ferment lactate is almost completely lost, although they are still able to ferment pyruvate. The addition of lipoic acid to such cell suspensions restores their ability to ferment lactate without affecting pyruvate dissimilation. The amount of carbon dioxide produced from the lactate carboxyl carbon by washed cell suspensions is stoicheometrically dependent upon the amount of exogenous lipoic acid added. Under these conditions lipoic acid appears to be functioning as a net electron acceptor from lactate.

The capacity of resting cells to incorporate carbon dioxide into acetate is qualitatively unaffected by prior extensive washing of the cells when pyruvate serves as the substrate. This suggests that lipoic acid is not required for carbon dioxide incorporation into acetate.

Resting cells of a glucose adapted strain of B. rettgeri, which does not require lipoic acid for growth, are unable to ferment lactate but will ferment pyruvate. The pyruvate fermentation is quite different from that carried out by lactate adapted cells. The glucose cells effect a dismutation of pyruvate with the formation of acetate, carbon dioxide and lactate.

Proposed Course of Projects:

Studies now in progress are designed to assess the precise role of lipoic acid in the conversion of lactate to pyruvate. Although present information has led to the tentative conclusion that lipoic acid is not involved at the substrate level, a purification of the system will be undertaken in an effort to gain more information concerning this point.

Part B Included NO

Serial No. NHI-118
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Metabolism of Sulfonium Compounds

Principal Investigator: Conrad Wagner

Technical: K. Chaney

Man Years (1960)
Total:
Professional:
Other:

Project Description:

Objectives: To study the metabolic pathway by which sulfonium compounds are degraded by microorganisms.

Methods and Major Findings:

An anaerobe, capable of growing on dimethyl-β-propiothetin as a major source of carbon has been isolated in pure culture from soil by the enrichment culture technique. Conditions for growing this microorganism in substantial yields have been developed.

During growth, three moles of dimethyl-β-propiothetin are converted to two moles of propionic acid andlmole of acetic acid. The sulfur containing product is principally, if not exclusively, dimethyl sulfide. Suspensions of washed cells carry out a similar decomposition of the dimethyl-β-propiothetin. The fermentation balance for the degradation of the sulfonium compound may be tentatively written as follows:

$$3(CH_3)_2\overset{+}{S}\text{-}CH_2\ CH_2\text{-}COOH + 2H_2O \longrightarrow 2CH_3\text{-}CH_2\text{-}COOH + 3(CH_3)_2S + CO_2 + 3H^+$$

Cell free extracts also have been obtained which are capable of carrying out the degradation of the dimethyl-β-propiothetin. The products of degradation obtained by the cell free system have not yet been identified.

Part A cont'd.

A new analytical method for measuring small amounts of sulfonium compounds has been developed to permit the measurement of dimethyl-β-propiothetin disappearance in small reaction mixtures. The range of this method is from 0.02 to 0.5 μMoles.

Proposed Course of Research:

The quantitative estimation of all the products of the degradation of dimethyl-β-propiothetin by intact cells must be completed. Identification and estimation of the products obtained in the cell free system must also be carried out. In addition, the products of the fermentation suggest that a propionic acid type of fermentation similar to the type found in Clostridium propionicum may be taking place. Confirmation of this type of metabolic pathway will be sought.

Part B Included NO

Serial No. NHI-119
Laboratory of Cellular Physiology
Section on Enzymes
National Heart Institute
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: General Title: Enzymatic Reaction Mechanisms.

(1) Mechanism of enzymatic isomerization of a primary to secondary alcohol coupled to phosphate elimination.

(2) Enzymes of microbial transsulfuration and the mechanism of enzymatic γ-elimination.

(3) Mechanism of enzymatic isomerization of methylmalonyl coenzyme A.

(4) Pyruvic kinase - fluorokinase mechanisms.

(5) Role of lipoic acid in lactate fermentation by Butyribacterium rettgeri. (Separate report by Dr. C. Wittenberger.)

Principal Investigator: Martin Flavin

Professional: Charles Wittenberger

Technical: Clarence Slaughter
George Hunt

Project Description:

Objectives: The general objective of our research is to study the mechanisms of a number of novel or complex enzymatic reactions at an increasingly detailed level, with the initial goal of approaching the level at which related organic reactions have been elucidated. In the early stages this program involves mainly the detailed study of reaction between substrates and coenzymes; of the latter we are currently concerned with pyridoxal phosphate, vitamin B_{12} coenzyme and lipoic acid. In addition, we are studying 2 novel reactions of phosphate: The enzymatic phosphorylation of inorganic fluoride, and the non-hydrolytic elimination of phosphate from a primary ester.

Major Findings:

The mechanism previously proposed for an enzymatic alcohol isomerization coupled to phosphate elimination

Part A cont'd.

(phosphohomserine - threonine) has been confirmed. When this reaction is carried out in 100% D_2O, exactly 2 solvent hydrogen atoms appear in newly formed threonine. A complete hydrogen degradation of threonine has been developed, and has shown that in all cases solvent hydrogen is incorporated only into the α and γ positions. The small incorporation of solvent H^3 previously reported reflects addition of protons to a double bond 40 times faster than tritium ions. The study of the mechanism of this unique reaction is still at an early stage. While the coenzyme (pyridoxal-P) can be formulated as potentiating removal of phosphoric acid, shifting of double bonds, and oriented addition of water, neither it nor phosphate can as yet be invoked to account for the different products in the reactions: homoserine → α-ketobutyrate, phosphohomoserine → threonine.

Two cleavage reactions (yielding keto acid and thiol) and 2 condensing reactions (between thiol and alcohol) have been proposed for many years as the basis for microbial transsulfuration. While each of the 4 reactions is predicted to be thermodynamically irreversible, the over-all process is reversible in micro-organisms (cysteine = homocysteine); its irreversability in animals (hcys → cys)has been attributed to absence of 2 of the 4 enzymes. However, transsulfuration, and the condensing reaction from hser, have never been demonstrated in vitro, nor have we been able to show cystathionine formation from hser + cys with various preparations of mutant and wild strains of Neurospora. Recently, some evidence has suggested the possibility that transsulfuration may be mediated by 2, rather than by 4, enzymes. Should this be the case, the dietary requirement for methionine in animals might reflect a curious reciprocal modification of the 2 enzymes, or it might indicate that control of transsulfuration rests on factors other than enzymes. We have accordingly begun the isolation of the 2 cleavage enzymes from Neurospora mutants believed to respectively lack the abilities to form cys and hcys. One of the enzymes (cystathionine → α-ketobutyrate + cys) has been purified 800 fold. More slowly, it appears also to catalyze hser → α-ketobutyrate and cys → pyruvate. Qualitative differences between it and the homologous crystalline liver enzyme are already apparent. Our immediate goal is to compare the mechanism of cystathionine → α-ketobutyrate with that already established for phosphohomoserine → threonine.

Investigation of the mechanism of enzymatic isomerization of methylmalonyl coenzyme A to succinyl CoA (annual report 1954-1955), has been hampered by lack of any model reaction in organic chemistry. Recently, however, the rearrangement of α-cinenic acid to geronic acid has been shown to involve a carboxyl-group transfer (the only case of transcarboxylation, to our knowledge). The further demonstration that the rearrangement was actually a transcarbonylation, mediated by elimination of carbon monoxide

Part A cont'd.

and its recapture by a resonance-stabilized carbonium ion, has prompted a reinvestigation of the methylmalonyl CoA isomerase mechanism, particularly since the B_{12} coenzyme for the enzymatic reaction could serve as a carbon monoxide carrier through its cobalt moiety. With enzyme preparations from lamb kidney entirely dependent on the addition of B_{12} coenzyme, we looked for formation of ^{14}CO from succinyl-1,4-C^{14} CoA, or its incorporation into the latter in the presence of methylmalonyl CoA. The results were negative.

A continuing problem is reparsented by our studies of the pyruvic kinase mechanism, particularly from the point of view of reconciling the abilities of the enzyme to phosphorylate alternately an enolic oxygen, or inorganic fluoride in the presence of bicarbonate. Perhaps the narrowest way in which these dissimilar reactions could be reconciled would be to place F^- on the enzyme surface in place of $C{=}C{-}O^-$, and $CO_3^=$ in place of $-CO_2^-$, and further to propose an intermediary acyl phosphate in both reactions. Early this year we carried out some experiments to test this hypothesis, by incubating either pyruvate or phosphoenolpyruvate with crystalline pyruvic kinase in ^3H_2O, in absence of nucleotides. The enzyme was found to catalyze a slow exchange of solvent hydrogen into pyruvate, but none into phosphoenolpyruvate. This result constitutes evidence against the hypothesis.

Proposed Course of Projects

Detailed plans for individual projects have been discussed above under "Major Findings." It is expected that current studies of the intimate details of coenzyme potentiation of biological catalysis will gradually tend in the direction of problems in the mechanism of enzyme action.

Part B included YES

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Publications

1. Martin Flavin and Clarence Slaughter, Mechanism of enzymic formation of threonine from O-phosphohomoserine. Biochim. Biophys. Acta, 36, 554-559 (1959).

2. Martin Flavin and Clarence Slaughter, Rapid and Specific determination of threonine, Anal. Chem. 31, 1983-1987 (1959).

3. Martin Flavin and Clarence Slaughter, Mechanism of enzymatic isomerization of a primary to secondary alcohol coupled to phosphate elimination. Federation Proc. 19, 48-52 (1960).

4. Martin Flavin and Clarence Slaughter, Purification and Properties of threonine synthetase of Neurospora. J. Biol. Chem. 235, 1103 (1950).

5. Martin Flavin and Tetsuro Kono, Threonine synthetase mechanism: studies with isotopic oxygen. J. Biol. Chem. 235, 1109-1114 (1960).

6. Martin Flavin and Clarence Slaughter, Threonine synthetase mechanism: studies with isotopic hydrogen. J. Biol. Chem. 235, 1112-1116 (1960).

In Press

7. Martin Flavin and Clarence Slaughter, Methylmalonyl Coenzyme A isomerase: investigation of a carbon monoxide transfer mechanism. J. Am. Chem. Soc. (February 1961).

8. Martin Flavin, Threonine synthetase from Neurospora. Methods in Enzymology, Vol. V, article 127a.

9. Methylmalonyl CoA. Martin Flavin, Methods in Enzymology, Vol. VI, article 209.

10. Martin Flavin, Determination of threonine with alcohol dehydrogenase. Methoden der Enzymatischen Analyse, Verlag Chemie.

Serial No. NHI-120
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Enzymatic Mechanisms of Biochemical Processes.
(1) The metabolism of propionic acid.
(2) The metabolism of heterocyclic compounds.
(3) Regulation of Biosynthetic Pathways.

Principle Investigator: E.R. Stadtman

Professional: P.Z. Smyrniotis
H. Eggerer, F. Lynen and P. Overath (from the Max Planck Institute fur Zellchemie, Munchen Germany)
G.N. Cohen (Pasteur Institute, Paris, France)

Man Years (1960)
Total:
Professional:
Other:

Project Description:

Objectives: It is the ultimate objective of this research to identify and characterize the individual enzymatic steps involved in the biosynthesis and degradation of various compounds of biological importance. Attention is directed to the metabolism of those substances which are poorly understood and which offer unique opportunities to study specific biochemical phenomena of fundamental interest and significance.

Major Findings:

(a) Propionate metabolism: In our previous studies evidence was obtained showing that the metabolism of propionate involves enzymatic steps in which biotin and vitamin B_{12} Co-enzyme are Co-catalysts. Biotin was shown to be implicated in the carboxylation of propionyl CoA to form methylmalonyl CoA and vitamin B_{12} Co-enzyme was shown to be necessary for the isomerization of methylmalonyl CoA to succinyl CoA. Evidance has now been obtained showing that the latter interconversion involves

Serial No. NHI-120
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Enzymatic Mechanisms of Biochemical Processes.
(1) The metabolism of propionic acid.
(2) The metabolism of heterocyclic compounds.
(3) Regulation of Biosynthetic Pathways.

Principle Investigator: E.R. Stadtman

Professional: P.Z. Smyrniotis
H. Eggerer, F. Lynen and P. Overath (from the Max Planck Institute fur Zellchemie, Munchen Germany)
G.N. Cohen (Pasteur Institute, Paris, France)

Man Years (1960)
Total:
Professional:
Other:

Project Description:

Objectives: It is the ultimate objective of this research to identify and characterize the individual enzymatic steps involved in the biosynthesis and degradation of various compounds of biological importance. Attention is directed to the metabolism of those substances which are poorly understood and which offer unique opportunities to study specific biochemical phenomena of fundamental interest and significance.

Major Findings:

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Part A cont'd.

an intramolecular rearrangement in which the thiolester carbon of methylmalonyl CoA migrates from the α carbon atom to the methyl carbon. This discovery eliminates from consideration the idea advanced by others that the isomerization involves intermolecular transcarboxylation reactions.

(b) Metabolism of riboflavin. Studies on the microbial degradation of riboflavin have been continued as part of a general program to determine more about the intermediary metabolism of heterocyclic compounds. The isolation of a bacterium capable of catalyzing the decomposition of riboflavin to CO_2 and NH_3 has been reported. In addition to riboflavin, this organism requires cosein hydrolyate for growth. In order to facilitate further studies on the metabolism of this bacteria the components present in casein hydrolysate that are needed for growth have been identified. They are methionine, valine, tyrosine, lysine, isoleucine and threonine. It has been established further that ribose, but not ribitol, will serve as the energy source.

(c) Regulation of Biosynthetic Pathways: Regulation of diverse biosynthetic pathways by changes in concentrations of the ultimate products is achieved by two distinct mechanisms: (1) a repression of the synthesis of enzymes involved in the metabolic pathway, and (2) specific inhibition of the activity of the first enzymatic step in the metabolic sequence. The first mechanism has been referred to as "repression" and the second is known as "feed-back" control.

In E. coli aspartokinase catalyzes the first step in a metabolic sequence leading ultimately to the formation of the three different amino acids, threonine, lysine and methionine. Its control by either of the above regulatory processes therefore presents a unique situation in which excessive production of one of these amino acids could lead to decreased aspartokinase activity and consequently to a deficiency in the production of the other two amino acids.

Evidence has been obtained showing that in E. coli a partial solution to this delemna is obtained through the synthesis of two different aspartokinases which are independently controled by the concentrations of L-lysine and of L-threonine. One enzyme is specifically and non-competitively inhibited by L-lysine and its formation is completely repressed when the organism is grown in the presence of 10^{-2} M L-lysine. The other aspartokinase is specifically and competitively inhibited by L-threonine but its concentration is not significantly influenced by growth in the presence of L-threonine. The biological implications of this discovery are supported by the further observation that in yeast, where the synthesis of lysine does not involve aspartate as a precursor the lysine sensitive aspartokinase is virtually absent

Part Cont'd.

here the threonine sensitive enzyme is the major if not the only enzyme present.

Proposed course of project:

Efforts will be made to determine if the biosynthesis of compounds derived from common precursors occurs by way of separate, parallel pathways or if a common branching pathway is involved. The studies on various aspects of propionate and riboflavin metabolism will be continued. In addition new studies on the biosynthesis and degradation of nicotinic acid, will be initiated to test the hypothesis that methylmalonyl CoA is an intermediary in this metabolism. Studies to ellucidate the mechanism of aromatic hydroxylation the synthesis of unsaturated fatty acids, enzyme induction and the reduction of isolated hydroxyl groups will be undertaken.

Part B Included YES

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

1. E.B. Brown Jr. and E.R. Stadtman Nucleotidases of Clostridium propionicum . I Purification and properties of Ferrous iron-dependent Nucleotide Monophosphatases and Pyrophosphatases, J.Biol. Chem. 235 2928-2934, 1960.

2. E.R. Stadtman, J.M. Earl, and E.B. Brown, Jr., Nucleotidases of Clostridium propionicum. II Role of Electron Transport Systems in Activation of a Diphosphopyridine pyrophosphatase, J.Biol. Chem. 235, 2935-2938, 1960

3. H.Goldfine and E.R. Stadtman, V. The Conversion of -alanine to Propionic Acid by Cell-free Extracts of Clostridium propionicum J.Biol. Chem. 235, 2238-2245, 1960.

4. E.R. Stadtman, P.Overath, H.Eggerer, and F. Lynen, The Role of Biotin and Vitamine B_{12} Co enzyme in Propionate Metabolism. Biochem. Biophys. Research Com. 2, 1, 1960.

5. E.R. Stadtman, P. Overath, H. Eggerer and F. Lynen, The Function of Biotin and Vitamin B_{12}-Co-enzyme in the oxidation of Fatty acids with an Uneven Number of Carbon Atoms. Proceedings, Symposium on Drugs Affecting Lipid Metabolism, Milan, June, 1960. (in press).

6. H. Eggerer, P. Overath, F. Lynen and E.R. Stadtman, On the Mechanism of the Cobamide Coenzyme Dependent Isomerization of Methyl Malonyl CoA to succinyl CoA, J.Am. Chem. Soc. 82 2643 (1960).

Honors:

Appointed Executive Editor of Archives of Archives of Biochemistry and Biophysics.

Appointed. Editor of Journal of Biological Chemistry.

Serial No. NHI-121
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Fatty Acid Synthesis by Adipose Tissue

Principal Investigator: M.G. Horning

Professional: D.B. Martin
P.R. Vagelos

Technical: Virmetta Gilmore
E.A. Williams
Maggie Earle

Man Years (1960)
Total:
Other:
Professional:

Project Description:

Objectives: The objective of this research is to determine the enzymatic mechanisms involved in the biosynthesis of long chain fatty acids of all types (saturated, unsaturated and branched.) This information should be useful in studying lipid metabolism in the various diseased states where fat metabolism is apparently not normal.

Major Findings: A fatty acid synthesizing system has been isolated from the epididymal fat pads of the rat which can make saturated fatty acids of the normal, odd numbered, iso- and anteiso type. These results are discussed in the report of Dr. P.R. Vagelos.

Proposed Course of Project: Controlling mechanisms in fatty acid synthesis will be studied using the purified mammalian enzyme system. This will involve primarily a search for hormonal effects on the enzyme. A study is being initiated in both mammalian tissues and tetrahymena pyriformis on the biosynthesis of unsaturated fatty acids.

Part B Included YES

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Publications:

1. Horning, M.G., Martin, D.B., Karmen, and Vagelos, P.R., Synthesis of Branched-Chain and Odd-Numbered Fatty Acids from Malonyl-CoA, Biochem. and Biophys. Res. Comm., 3, 101-106 (1960).

2. Horning, M.G., Martin, D.B., Karmen, A., and Vagelos, P.R., Enzymatic Synthesis of Branched-Chain and Odd-Numbered Fatty Acids, J.Biol. Chem. in press.

3. Martin, D.B., Horning, M.G., and Vagelos, P.R., Fatty Acid Synthesis in Adipose Tissue: Purification and Properties of a Long Chain Fatty Acid Synthesizing System, J. Biol. Chem., in press.

4. Horning, M.G., Martin, D.B., Karmen, A., And Vagelos, P.R., Methods of Lipid Analyses and Their Use in the Study of Fatty Acid Metabolism, Symposium on Drugs Affecting Lipid Metabolism, Milan, 1960, in press.

5. Vagelos, P.R., W.J.A. VandenHeuvel, and M.G. Horning, Identification of Hydroxamic Acids by Gas Chromatography of Isocyanate Derivatives. Analytical Biochemistry, in press.

Serial No. NHI-122
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of Hormonal and Drug Effects on the Mobilization and Transport of Fat

Principal Investigator: M.G. Horning

Technical: Virmetta Gilmore
Elizabeth A. Williams
Maggie Earle
K. Chaney

Cooperating Unit: Dr. H. Maling (Lab. of Chem. Pharm.)
Dr. Masao Wakabayashi
Mr. Arthur Frank
Mr. William Butler

Project Description:

Objectives: The purpose of this research is to study the regulation of fat mobilization and transport by hormones and drugs in the intact animal, and then to extend these observations to the subcellular level in order to study the particular enzymatic reactions which have been affected by these pharmacologically active compounds.

Major Findings: Origin of the fatty acids in the triglyceride fatty livers: The technique of gas chromatography has been applied as a new approach to this problem. Since the rat cannot synthesize linoleic acid, this acid can be used a non-radioactive tracer to determine if fatty acids have been synthesized and/or transported from fat depots. Analyses of the fatty acid composition of the liver and adipose tissue triglycerides indicated that the deposited liver triglycerides had the same percentage composition of linoleic acid as the adipose tissue. There was no depletion of the linoleic acid in the liver phospholipids, and a labelling experiment showed that under the influence of alcohol, the rat had not synthesized linoleic acid. The total amount of linoleic acid in the liver triglycerides by transport from the fat depots.

The results obtained by gas chromatographic analyses of liver and adipose tissue triglycerides after treatment with ethionine and carbon tetrachloride were similar. The linoleic acid content of the deposited triglycerides in the liver indicated

Part A cont'd.

transport from the depots. However, analyses of the phospholipid fatty acids were of interest. There had been no significant change in the absolute amount of phospholipids, but rather striking changes had occurred in the fatty acid composition of the phospholipids.

Proposed Course of Project:

The changes in the fatty acid composition of the phospholipids will be studied in greater detail. The various classes of phospholipids will be separated and the fatty acid composition determined by gas chromatography to determine if the changes in composition are associated with a particular type of phospholipid.

The Effect of Alcohol in the Enzymatic Incorporation of Palmitic Acid-1-C^{14} into Liver Lipid Fractions: The pattern of incorporation of radioactive palmitic acid into the various lipid fractions by cell free homogenates from livers of normal rats and rats pretreated with ethanol has been studied by Dr. Wakabayaski. Preliminary results are very promising since the pattern of incorporation by the two types of homogenates shows quite interesting differences. This approach may provide information on the mechanism responsible for the accumulation of triglycerides in the liver after ethanol and can be extended to other pharmacologically active compounds.

The Effect of CCL -poisoning on the Release of Triglycerides from Rat Liver: The possibility was investigated that very high levels of liver triglycerides found inrats treated with CCl_4 might be due in part to a block in the removal of triglycerides from the liver. (It is generally assumed that fat is carried to the liver in the form of free fatty acids, and is removed from the liver as triglycerides.) The removal of triglycerides from the liver was studied by following the incorporation of intravensously injected radioactive palmitic acid into plasma triglycerides. The plasma lipids were fractionated and the radioactivity in each fraction was determined at 10, 20, 30, 40 and 60 minutes after injection of the labeled palmitate. In normal fed and fasted rats a peak in the radioactivity of the triglycerides was observed; in rats treated with carbon tetrachloride no such peak was observed. There was no significant difference in the radioactivity of the plasma free fatty acids of the normal and CCl_4 treated rats, and there was no inhibition of incorporation of radioactive palmitic acid into the triglycerides of the liver of the CCl_4 treated rats. This block in the removal of triglycerides from the liver of carbon tetrachloride rats may be partially responsible for the excessive amounts of triglycerides in the liver.

Part A cont'd.

Proposed Course of Project:

The effect of adrenergic and ganglionic blocking agents abd hypophysectomy on the incorporation of radioactive palmitic acid into the various lipid fractions by liver homogenates of normal, alcohol treated and CCl_4 treated rats will be studied.

Part B Included YES

Part B

1. Brodie, B.B., W.M. Butler, Jr., M.G. Horning, R.P. Maickel, and H.M. Maling, Alcohol-induced triglycerides triglycerides deposition in liver through derangement of fat transport. Am. J. Clin. Nutrition,

2. Brodie, B.B., H.M. Maling, M.G. Horning and R.P. Maickel. The Triglyceride deposition in rat liver through derangement of fat transport by alcohol and other chemical agents. Symposium of Drugs Affecting Lipid Metabolism, Milan, 1960, in press.

3. Butler, W.M., Jr., H.M. Maling, M.G. Horning and B.B. Brodie, The direct determination of liver triglycerides, J. Lipid Research, in press.

4. Horning, M.G., E.A., Williams, and E.C. Horning, Separation of Tissue cholesterol esters and triglycerides by silicic acid chromatography. J. Lipid Research, in press.

5. Popjak, G., L. Gosselin, Irene Youhotsky Gore, A. DeWaard, N.L.R. Bucher, and Marjorie Horning, Studies on Biosynthesis of Squalene and Cholesterol from Mevalonic Acid., "Biochemistry of Lipids", Pergamon Press (1960, p. 163-172.

6. Horning, M.G., E.A. Williams, H.M. Maling, and B.B. Brodie, Depot Fat as Source of Increased Liver Triglycerdes after Ethanol, Biochem. Biophys. Res. Comm., 1960 in press.

Serial No. NHI-123
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: (1) Metabolism of N-onium compounds and methyl donor compounds in anaerobic microbes.

Principal Investigator: Hugh R. Hayward

Technical: Percy Ricketts

Man Years (1960)
Total:
Professional:
Other:

Project Description

Objectives: The general objectives of the project has been to study the fermentation of choline by cell-free extracts of Vibrio cholinicus with a view to elucidating intermediates and reaction mechanisms and to studying the sites of phosphate esterification in the overall fermentation.

Major Findings

Acetaldehyde as an intermediate in the fermentation of chloine. Experiments with C^{14}-labeled choline showed that acetaldehyde is formed from the 2-carbon moity of choline during conversion of the latter to ethanol and acetate by extracts of V. cholinicus. Further, extracts of the organism catalyze the production of ethanol and acetate from acetaldehyde at least as rapidly as from choline.

For maximal activity for the dismutation of acetaldehyde by these extracts it was shown that glutathione, ferrous iron, TPN, ADP and CoASH are required. Associated with the reaction is an esterification of inorganic phosphate. The system is inhibited by several "sulfhydryl inhibitors," but is not inhibited by 2,4-dinitrophenol under conditions which cause profound inhibition of choline degradation.

Part A cont'd.

Individual Enzymes of Acetaldehyde Metabolism.

The alcohol dehydrogenase has been purified 65-fold by a combination of high speed centrifugation, protamine treatment, ammonium sulfate fractionation and heat treatment. Study of this partially purified preparation revealed an absolute requirement for TPN (DPN would not substitute), but no other cofactors could be demonstrated; specifically ferrous iron has no effect.

The aldehyde dehydrogenase was purified approximately 20 fold over the crude extracts by centrifugation, protamine and salt steps, acetone precipitation and by chromatography on DEAE cellulose. Since CoASH is required for maximal acitivity for the overall dismutation, it was postulated that the oxidation of acetaldehyde proceeded through acetyl-SCoA via a CoASH-linked dehydrogenase. However, the system has not yet been resolved for CoASH; there is an absolute requirement for a -SH compound, and CoASH appears to be the most effective source on a molar-SH basis; but attempts to demonstrate the thiol ester have been negative. Attempts to demonstrate the oxidation of TPNH with acetyl-SCoA have been likewise unsuccessful. As with the alcohol dehydrogenase, there is absolute specificity for TPN. This enzyme is inhibited by iodoacetate and by p-chloromercuriphenylsulfonate but not by 10^{-2} M arsenite.

Phosphotransacetylase activity has been demonstrated in crude extracts and in the 55-90% saturated ammonium sulfate fraction; no detailed studies on this enzyme have been carried out as yet.

An acetokinase is present in crude extracts and has been partially purified by salt fractionation. As yet only preliminary experiments have been conducted with this enzyme.

Thus there are present all four enzymes necessary for the following pathway of acetaldehyde metabolism: dismutation to ethanol and acetyl-SCoA, transfer of the acyl group to phosphate forming acetyl phosphate, and finally formation of ATP and free acetate. However, conclusive proof that this is the pathway of acetaldehyde oxidation in this organism is not yet at hand.

Fermentation of choline by an unidentified Clostridium.

Recently a contaminant appeared in autoclaved growth medium for V. cholinicus. The organism was isolated, identified as a member of the genus Clostridium, and conditions for growth

Part A cont'd.

established. Fermentation balances in growing cultures with choline as substrate (as yet incomplete) reveal some considerable variation in products. Thus in one case acetaldehyde and trimethylamine were the major products. In other cultures trimethylamine ethanol, and acetate are the principal products. Of more interest, however, is the production of ammonia (indicating some demethylation reaction) in certain cultures, with the concomitant production of butyrate and propionate as well as three other unidentified volatile acids. Preliminary studies indicate that the butyrate arises from the 2-carbon moity of choline, but the fate of the methyl groups is still unknown.

Proposed course of project:

In the coming year, studies will be carried on in the following areas:

1. Further investigation of the enzymes of acetaldehyde metabolism in extracts of V. cholinicus with particular emphasis on establishing the immediate product of aldehyde oxidation.

2. Study of the earlier parts of the pathways of choline fermentation in V. cholinicus. Of particular interest is the matter of whether or not betaine aldehyde is an intermediate and the nature of the cleavage reaction.

3. Investigation of the fermentation of choline in the newly isolated clostridium with particular interest in the mechanism and products of demethylation.

Part B Included YES

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Publications:

1. Hugh R. Hayward and Thressa C. Stadtman, Anaerobic Degradation of Choline II. Preparation and Properties of cell-free Extracts of Vibrio cholinicus, J. Biol. Chem., 235, 538-543, 1960.

2. Hugh R. Hayward, Anaerobic Degradation of Choline III. Acetaldehyde as an Intermediate in the Fermentation of Choline by Extracts of Vibrio cholinicus, J. Biol. Chem., December, 1960 (in press).

Serial No. NHI-124
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Metabolism of Heterocyclic Compound

Principal Investigator: Lin Tsai

Man Years (1960)
Total:
Other:
Professional:

Project Description:

Objectives: The purpose of the research is to attempt the isolation and characterization of intermediates in the metabolism of heterocyclic compounds and to synthesize compounds for use in the study of some enzymatic reactions and their mechanisms.

Major Findings: It has been established (see annual report of 1959) that the bacterial degradation of 1-ribityl-6,7-dimethyl-1,2,3,4-tetrahydro-2,3-diketoquinoxaline proceeded with the loss of the ribityl group to yield 6,7-dimethyl-2,3-quinoxalinediol as one of the major products. It is of great interest to gain some knowledge concerning the fate of the ribityl side-chain in this transformation. In order to study this aspect of the problem, a substrate containing a labelled carbon atom in the ribityl group is needed, hence the synthesis of 1-ribityl-6,7-dimethyl-1,2,3,4-tetrahydro-2,3-diketoquinoxaline-1'-^{14}C was undertaken. The synthesis of this labelled compound has been achieved by the following sequence of reactions: (a) condansation of 3,4-dimethylaniline with d-ribose-1-C^{14} under reductive conditions to furnish N-ribityl-3,4-dimethylaniline-1-^{14}C, (b) coupling of the N-ribityl compound with benzenediazonium chloride to give a diazo compound; and (c) catalytic hydrogenation of the diazo compound in the presence of diethyl oxalate to yield the desired labelled compound. One other approach to the synthesis of this compound was also studied in detail but the product was in general a complex mixture.

The final stage in the bacterial degradation of riboflavin involves the conversion of 6,7-dimethyl-2,3-quinoxalinediol to 3,4-dimethyl-α-pyrone-6-carboxylic acid and oxamide, for the purpose of studying the mechanism of this reaction, attempts to

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Part A cont'd.

prepare two plausible intermediates, 6,7-dimethyl-2,3,5-quinoxalinetriol and 3,4-dimethyl-α-pyrone-6-carboxylic oxamide, were made.

For the preparation of the quinoxalinetriol the reaction sequence: $R-NO_2$ —— $R-NH_2$ —— R-OH was employed so as to introduce the extra hydroxyl group to the quinoxaline molecule. Direct nitration of 6,7-dimethyl-2,3-quinoxalinediol was studied under various conditions but on no occassion could a pure nitro derivative be isolated. Although nitration did occur the products obtained were invariably complex mixtures of no practical value. Nitration of 6,7-dimethylquinoxaline was also unsuccessful. Attention was directed to the nitration of N,N-diacetyl-4,5-dimethyl-1,2-phenylenediamine. A mono-nitro compound was indeed obtained, but the hydrolysis of this compound to the diamino derivative took an unexpected course. Instead of the diamine, hydrolysis of the nitro compound gave a benzimidazole derivative through a cyclization process. This sequence of reactions constitutes a convenient method for the preparation of a new compound, 4-nitro-5,6-dimethylbenzimidazole.'

Model experiments leading to the synthesis of monoacyl derivatives of oxamide were carried out. Condensation reactions between an acyl chloride and oxamide; an amide and ethyl oxalyl chloride; an acyl chloride and oxamethane; and ethyl oxalyl chloride and benzamidine were studied. None has as yet provided any oxamide derivatives of the desired structural feature.

Proposed course of project

Present studies will be continued.

Serial No. NHI-125
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A study of the effect of various metabolites on cellular differentiation and the rate of protein synthesis of Dictyostelium discoideum.

Research Staff: Professional: Micah Krichevsky
Technical: K. Chaney

Man Years (1956)
Total:
Professional:
Other:

Project Description:

Objective: (a) To further characterize the stimulatory effect of histidine and the inhibitory effect of adenine on the rate of differentiation. (b) To determine and characterize the ability of metabolites related to those in (a) above to affect differentiation.

Major Finding:

Histidine increased the rate of differentiation by as much as 2-fold at an optimum concentration of 0.2-0.5 M. The rate of C^{14} labeled mixed amino acids incorporated into protein (taken as a measure of protein synthesis) also was increased by the presence of unlabeled histidine. The stimulation was maximal at preculmination. The inhibition of differentiation by adenine could be overcome by histidine. Glucose stimulated differention and was even more effective when combined with histidine. Glucose apparently stimulated protein synthesis maximally at the aggregation stage. The glucose effect on protein synthesis was essentially nullified by the presence of histidine.

Two amino acids which increase the rate of respiration (glycine and cysteine) also stimulated both differention and protein synthesis either alone (i.e., combined but without histidine) or, to a greaterextent, in the presence of histidine. Phenylalanine does not stimulate respiration and did not affect differentiation. Histidine and asparagine do not

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Part A cont'd.

stimulate respiration but do stimulate differentiation.

It is tentitively concluded that histidine is not unique in stimulating either differentiation or protein synthesis, and that it is not acting as a prime energy source nor is it simply limiting for protein synthesis.

Part B Included NO

Serial No. NHI-126
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Biosynthesis of Phenazine-1-carboxylic Acid.

Principle Investigator: Mark Levitch

Technical: K. Chaney

Man Years (1960)
Total:
Professional:
Other:

Project Description:

Objectives: To define the biosynthetic pathways involved in the production of phenazine-1-carboxylic acid by Pseudomonas aureofaciens. The following general approaches have been employed: (1) incorporation of radioactive substrates (2) chemical inhibition studies (3) mutant studies.

Major Findings:

Earlier studies had established conditions for pigment production by preparations of washed cells, and the stability of these cells in the frozen state for periods of up to several months duration. Simple media for the production of pigment had been defined. A number of radioactive compounds were added to media of growing cultures, and the incorporation of radioactivity into phenazine-1-carboxylic acid determined. The specific radioactivity of the pigment resulting from these experiments varied from 10^{-3} to 10^{-5} microcuries per micromole. In addition, pigment production was shown to be inhibited by addition of p-aminobenzoic acid or o-phenylenediamine to the medium in experiments performed with suspensions of washed cells.

Subsequent studies in which radioactive substrates were added to washed cell preparations resulted in a much more efficient incorporation of radioactive label. The specific radioactivity of the phenazine-1-carboxylic acid produced in these experiments was in the range 10^{-2} to 10^{-3} microcuries per

Part A cont'd.

micromole, several orders of magnitude higher than the values obtained with actively growing cultures under much longer periods of incubation (12 hours for the former, 5 days for the latter).

The following substrates gave rise to labeled pigment in the washed cell experiments: Sodium Formate-C^{14}, Sodium Acetate-1-C^{14}, sodium DL-Lactate-1-C^{14}, DL-Alanine-2-C^{14}, Glycerol-1, 3-C^{14}, L Threonine-U-C^{14}, Glucose-6-C^{14}, Sodium Pyruvate-1-C^{14}, DL-Serine-3-C^{14}, DL Tyrosine-U-C^{14}, DL-Tryptophane-3-C^{14}. It is interesting to note that pigment produced in the presence of Glucose-1-C^{14} exhibited no radioactivity.

Attempts to demonstrate a precursor role for some of these substrates by the technique of isotopic competition have not been successful. Using radioactive glycerol-1,3-C^{14} in combination with unlabeled serine, alanine, or glycine, no decrease of specific radioactivity of the pigment was noted. However, preliminary examination of the aqueous medium after chloroform extraction (to remove the pigment) indicate labeling of some as yet undetermined ninhydrin-positive material.

A survey of the amino acids was made to determine the nitrogen sources most effective in the synthesis of phenazine-1-carboxylic acid. Using cells grown on any of several complex media, considerable variation was observed with respect to amino acids which most effectively served as nitrogen sources. These results may reflect variations in the endogenous pools of amino acids under the different growth conditions.

In collaboration with Dr. Lin Tsai, the feasibility of a systematic degradation of phenazine-1-carboxylic acid has been investigated further, as an extension of the radioactive labeling studies. The yield of the 1-amino derivative has been improved to 65% from the carboxylic acid. However, in order to conserve the biological product, 1-methoxyphenazine was synthesized by a known procedure in 33% yield. Attempts to oxidize these phenazine derivatives under a wide variety of conditions have yielded no recognizable products. Attempts of introduce additional activating groups by nitration and subsequent reduction have failed.

Some preliminary experiments were undertaken to produce mutants of P. aureofaciens in which the ability to synthesize phenazine-1-carboxylic acid has been impaired. No such mutant has been found. However, ultraviolet radiation of cultures has produced a strain with a higher yield of pigment, indicating that the organism may yet give rise to other mutants more useful in this investigation.

Part A cont'd.

A wide range of aromatic compounds have been found to inhibit pigment synthesis: o-anisidine, N-formylaniline, aniline, acetanilide, 2,3 dihydroxyquinoxaline, pyrogallol, o-aminophenol, o-phenylenediamine, anthranilic acid, chloramphenicol, novobiacin, Whether these inhibitions are general metabolic effects, or more specifically related to pigment synthesis is unknown. Spectrophotometric examination of media containing o-phenylenediamine indicates the formation of a compound or compounds with spectral characteristics unlike phenazine-1-carboxylic acid and o-phenylenediamine. However, time course studies indicate no relation to pigment synthesis. An inhibition of pigment synthesis by glucose has been noted in washed cell suspensions. Inhibition of pigment synthesis by metal ions has been found, but has not been investigated further. It is of interest to note the production of a compound with absorption maximum at 265 mμ with metal inhibition and the fact that under normal conditions of pigment synthesis the phenazine spectrum of extracts is accompanied by an additional maximum at 280 mμ.

Proposed Course of Project

Isotopic competition studies will be expanded, with a view toward the discovery of one or more intermediates in the biosynthetic pathway. An attempt will be made to accumulate and characterize the compounds absorbing at 265 and 280 mμ, and to define their relationships to phenazine-1-carboxylic acid. It is felt that a more thorough attempt to produce mutants blocked in pigment synthesis may also be fruitful.

Part B Included NO

Serial No. NHI-127
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Intermediary Metabolism of Amino Acids, with particular emphasis on those reactions involving overall reductive deaminations.

Research Staff: Professional:

Technical: J.K. Hardman

Man Years (1960)
Total:
Professional:
Other:

Project Description:

Objectives: To determine the enzymatic steps whereby amino acids are converted to fatty acids and ammonia by a balanced oxidation-reduction process with particular emphasis on those anaerobic electron-transfer reactions involved which may possibly be coupled to a phosphorylating mechanism.

Major Findings:

Additional information has been obtained substantiating the postulated series of reactions whereby γ-aminobutyrate is converted to ammonia and fatty acids (acetate and butyrate) by Clostridium aminobutyricum.

I. Overall REaction in Crude Sonic Extracts: Cofactor requirements for acetate production anaerobically from γ-aminobutyrate have been defined in crude dialysed extracts.

Carbon and isotope balance studies in crude dialysed extracts indicate that the overall reaction is similar to that in growing cultures of the organism except that the ratio of products is somewhat different, resulting in the accumulation of several of the intermediates, glutamate and γ-hydroxybutyrate.

II. Succinic Semialdehyde as an Intermediate: In dialysed extracts, there was an accumulation of succinic semialdehyde, identified, a) enzymatically with substrate specific dehydrogenase of Pseudomonas fluorescens;

Part A cont'd.

b) by the isolation and identification of its dinitrophenylhydrazone chromatographically and spectrally; and
c) by the isolation and identification of succinate following an incubation with above mentioned dehydrogenase.

Requirements for acetate production from succinic semialdehyde were defined. REsults indicate that a glutamic dehydrogenase functions by generating DPNH and by releasing free ammonia; and that the activating step occurs after succinic semialdehyde reduction.

III. γ-Hydroxybutyrate as an Intermediate: In dialysed extracts γ-hydroxybutyrate accumulated and was isolated and identified by chromatography of its hydroxamate.

Growth yields with γ-hydroxybutyrate and ammonia as substrates were equal (on basis of substrate molarity) to those with γ-aminobutyrate as substrate.

Iv. Suggestive Evidence for Electron-Transport Phosphorylation In studies carried out in other anaerobic fermentations wherein the enzymatic steps and the moles of ATP formed / mole of substrate utilized are known, it has been shown that there was a cell yield of 10 μg of cells (dry weight)/μmole of substrate utilized for every μmole of ATP formed. According to the proposed breakdown of γ-aminobutyrate, there could be formed at the substrate level 1 mole of ATP/2moles of γ-aminobutyrate fermented. Further energy, if available, must be obtained from electron-transfer processes.

Results of similar studies in growing cultures of this organism indicate that 1 mole of ATP is formed/1 mole of substrate (γ-aminobutyrate and γ-hydroxybutyrate) utilized. Therefore, electron-transport phosphorylation may occur in this organism after γ-hydroxybutyrate formation.

Proposed Course of Action

Further investigations will be attempted to establish the proposed compounds as intermediates in γ-aminobutyrate fermentation leading to the formation of vinylacetate and/or crotonate and to demonstrate directly a possible phosphorylating mechanism that may be coupled with crotonate reduction to butyrate.

Part B Included YES

Serial No. NHI-127
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Publications

1. John K. Hardman and Thressa C. Stadtman, Metabolism of w-Amino Acids. I Fermentation of γ-aminobutyric acid by Clostridium aminobutyricum N.Sp., J.Bacteriol 79 544-548 (1960).

2. John K. Hardman and Thressa C. Stadtman, Metabolism of w-Amino Acids. II. Fermentation of Δ-aminovaleric acid by Clostridium aminovalericum N.Sp., J. Bacteriol. 79 549-552 (1960).

3. John K. Hardman, γ-Hydroxybutyrate Dehydrogenase from Clostridium aminobutyricum, Methods in Enzymology, Vol. V, 1961 (in press).

Serial No. NHI-128
Laboratory of Cellular Physiology
Section on Enzymes
National Institutes of Health
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: (1) Energy-rich compound metabolism.
(2) Heterocyclic Compound Metabolism.

Principal Investigator: P.R. Vagelos

Professional: D.B. Martin
E. Kravitz
W. Sly
M.G. Horning

Technical: A. Alberts
K. Chaney
G. Henderson

Man Years (1960)
Total:
Professional:
Other;

Project Description:

Objectives: The objective of this research is to determine the enzymatic mechanisms of certain reactions involved in fatty acid metabolism and alkaloid biosynthesis. The study of the mechanism of biosynthesis of long chain fatty acids in particular deals with the enzymatic reactions that convert acetate to palmitate. Energy-rich compounds are involved as intermediates in a series of reactions that is not yet completely elucidated. A knowledge of the details of normal fatty acid metabolism may be useful in the study of pathological conditions in which lipid metabolism is known to be deranged. The study of heterocyclic compound metabolism deals in particular with the intermediate steps in the biosynthesis of alkaloids derived from poppy plants.

Major Findings: Malonyl CoA-CO_2 Exchange Reaction: The enzyme that catalyzes this reaction has been highly purified from extracts of C. kluyveri. The reaction requires malonyl CoA, $C^{14}O_2$, enzyme and any of the unsubstituted fatty acyl CoA derivatives. Although the product of the reaction has only been tentatively identified, the reaction being studied is probably:

cont'd.

Part A cont'd.

$$C^*OOHCH_2COSCoA + R\text{-}COSCoA \rightleftharpoons C^*O_2 + CoASH + RCOCH_2COSCoA$$

The condensed product of the reaction appears to be bound to the enzyme, a finding reported earlier by F. Lynen in yeast. After subjecting the purified enzyme that catalyzes this reaction to paper electrophoresis, it can be shown that two proteins are separated that are both required for the reaction.

Because of the likelihood that such a condensation reaction might be involved in long chain fatty acid synthesis, extracts of C. kluyveri were tested for the ability to make fatty acids from C^{14}-malonyl CoA. A completely soluble enzyme complex was demonstrated in a 65-95% ammonium sulfate fraction that requires acetyl CoA, malonyl CoA and TPNH as well as enzyme to make fatty acids. The predominant product of the reaction was stearate, although smaller amounts of C-16, C-20 and C-22 were also formed.

Fatty Acid Synthesis by Adipose Tissue: A fatty acid synthesizing enzyme system, consisting of submicrosomal particles, has been purified from epidydimal fat pads of rats by Drs. Martin and Horning. This enzyme complex requires acetyl CoA, malonyl CoA and TPNH to synthesize primarily palmitate, although smaller amounts of C-14 and C-18 are also made. The properties of this enzyme have been studied in detail including pH optimum, stoichiometry, and mercaptan requirement. Of especial interest was the fact that the synthetic enzyme could catalyze the malonyl CoA-CO_2 exchange reaction. In fact the two activities were concentrated together throughout a 60-fold purification. This suggests that the exchange reaction may be an integral reaction of fatty acid synthesis in both mammalian and bacterial systems. A study of the specificity of the fatty acid synthesizing enzyme system for fatty acyl CoA derivatives indicated that odd numbered and branched chain derivatives could substitute for acetyl CoA. In such cases odd numbered, iso, and anteiso long chain fatty acids were formed. The products of these reactions were identified by gas chromatography.

Propionate Metabolism: The enzyme that catalyzes the TPN specific dehydrogenation of malonyl semialdehyde CoA to malonyl CoA was purified up to 30-fold by Dr. Sly. However, further purification proved extremely difficult, and all attempts to separate this enzyme from β-hydroxypropionyl CoA dehydrogenase failed. For this reason the purification will be pushed no further.

Part A cont'd.

Alkaloid Biosynthesis: Dr. Kravitz demonstrated that several isotopes could be incorporated into alkaloids of the poppy plant by parts of plants and by crude homogenates. However, cell-free extracts could not synthesize alkaloids under his experimental conditions. The biosynthesized alkaloids from the radioactive experiments were separated by column chromatography and identified by paper chromatography.

Proposed Course of Project

The mechanism of the malonyl CoA-CO_2 exchange reaction with the involvement of two separate enzymes will be probed. The intermediate reactions beyond the condensation reaction in fatty acid biosynthesis will be studied in both C. kluyveri and mammalian tissue extracts. Controlling mechanisms in fatty acid synthesis will be studied using the purified mammalian enzyme system. This will involve primarily a search for hormonal effects on the enzyme. A study is being initiated in both mammalian tissues and tetrahymena pyriformis on the biosynthesis of unsaturated fatty acids.

Part B included YES

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Publications:

1. Vagelos, P.R. Propionic Acid Metabolism, IV. Synthesis of Malonyl Coenzyme A, J.Biol. Chem., 235, 346-360 (1960).

2. Vagelos, P.R. and Alberts, A.W., Synthesis of β-Ketooctanoyl Coenzyme A, Anal. Biochem. 1, 8-16 (1960).

3. Horning, M.G., Martin, D.B., Karmen, A., and Vagelos, P.R., Synthesis of Branched-Chain and Odd-Numbered Fatty Acids from Malonyl-CoA, Biochem. and Biophys. Res. Comm., 3, 101-106 (1960).

4. Vagelos, P.R. Acrylyl Coenzyme A Aminase, in S.P. Colowick and N.O. Kaplan (Editors), Methods, in Enzymology, New York, Academic Press, Inc., in press.

5. Vagelos, P.R., Preparation of Fatty Acyl Derivatives of Coenzyme A, ibid, Vol. VI, in press.

6. Vagelos, P.R., and Alberts, A.W., Malonyl CoA-CO_2 Exchange Reaction, J. Biol. Chem., 235, 2786-2791 (1960).

7. Vagelos, P.R., Acyl-S-CoA-compounds: Malonyl Semialdehyde-CoA, in H.U. Bergmeyer (Editor) Methoden der enzymatischen Analyse, Heidelberg, Verlag Chemie GmbH, in press.

8. Vagelos, P.R., Acyl-S-CoA-compounds: Acrylyl-CoA, ibid, in press.

9. Vagelos, P.R., Acyl-S-CoA-compounds: β-Hydroxypropionyl-CoA, ibid, in press.

10. Horning, M.G., Martin, D.B., Karmen, A., and Vagelos, P.R., Enzymatic Synthesis of Branched-Chain and Odd-Numbered Fatty Acids, J.Biol. Chem., in press.

11. Martin, D.B., Horning, M.G., and Vagelos, P.R., Fatty Acid Synthesis in Adipose Tissue: Purification and Properties of a Long Chain Fatty Acid Synthesizing System, J. Biol. Chem., in press.

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Individual Project Report
Calendar Year 1960

Part B cont'd.

Publications:

12. Horning, M.G., Martin, D.B., Karmen, A., and Vagelos, P.R., Fatty Acid Metabolism, Symposium on Drugs Effecting Lipid Metabolism, Milan, 1960, in press.

13. Vagelos, P.R., W.J.A. Vandenheuvel, and M.G. Horning Identification of Hydroxamic Acids by Gas Chromatography of Isocyanate Derivatives. Analytical Biochemistry, in press.

Serial No. NHI-129

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: See objectives

Principal Investigator: T.C. Stadtman

Professional:

Technical:

Man Years (1960)
Total:
Other:
Professional:

Project Description:

Objectives: (1) Metabolism of one-carbon compounds with special reference to the reaction mechanism involved in the reduction of carbon dioxide to methane.

(2) Participation in the research program of Dr. M. Grunberg-Manago (Institut de Biologie et Physico Chimique, Paris, France) on the biological role and properties of polynucleotide phosphorylase. Studies were made on the enzyme obtained from Clostridium sticklandii.

(3) Further studies on the ATP-forming glycine reductase system of C. sticklandii.

Major Findings

(1) From Jan. to April 1, 1960 experiments were continued with Dr. Axel Lenzius (in the laboratory of Prof. F. Lynen, Munchen, Germany) on the mechanism of carbon dioxide reduction to methane. Dried cell preparations and extracts of methanobacterium omelianskii were employed as experimental material. From earlier studies it was fairly well established that none of the simple one-carbon compounds are free intermediates in the overall conversion of Co_2, the most oxidized form of carbon to methane, its most reduced form (reaction sequence 1).

$$CO_2 \xrightarrow{+2H} HCOOH \xrightarrow{+2H} HCHO \xrightarrow{+2H} CH_3OH \xrightarrow{+2H} CH_4$$

$$\downarrow\uparrow \pm H_2O$$

$$Co$$

Part A cont'd.

Carbon monoxide, which is metabolized by all methane bacteria so far examined, and formate were the only compoonds of the above series which could conceivably be free intermediates. In our experiments, we could demonstrate that neither carbon monoxide nor formate are reduced with molecular H_2 by dried cell preparations of M. omelianskii under concitions where Co_2 is very rapidly reduced to CH_4.

Coenzyme analyses revealed that cells of two different species of methane bacteria are exceptionally high in biotin and in vitamin B_{12} coenzyme(s). These coenzymes are synthesized entirely from ethanol and/or carbonate in one species; entirely from formate in the other species and presumably are involved in the exaggerated metabolic process in question-namely Co_2 reduction to methane.

In dried cell systems an extremely oxygen-labile compound is synthesized during a lag period that is required for Co_2 reduction to CH_4 with molecular H_2. Both Co_2 and H_2 must be present to form this compound and once it reaches optimum concentration it allows CH_4 formation to proceed at a linear rate for hours or as long as the two substrates are available. This lag period is not associated with a deficiency in the hydrogenase system nor is it overcome by the addition of ATP or ATP-generating systems. Once the oxygen-labile compound is formed it can be kept for hours in an atmosphere of pure H_2 (in the absence of Co_2).

The enzyme system catalyzing Co_2 reduction to CH_4 is inhibited by a number of metabolic poisons, notably by low levels of 2,4-dinitrophenol. This may be taken as additional evidence suggesting coupled electrontransport phosphorylation during the overall reaction: $Co_2 + 4H_2 \text{ —— } CH_4 + 2H_2O$. In soluble enzyme preparations of M. omelianskii Co_2 is fixed into pyruvate and alanine by a dinitrophenol sensitive mechanism. This appears not to be due to a DNP-activated adenosine triphosphatase.

(2) From April 1, 1960 experiments conducted in the laboratory of Dr. Grunberg-Manago, Chef de Service Biochimique, C.N.R.S., revealed that the anaerobic bacterium, Clostridium sticklandii, also posseses a very active polynucleotide phosphorylase. This enzyme was purified somewhat and some of its properties ascertained. The phosphorylase could be separated from another enzyme also present that catalyzes an exchange reaction between orthophosphate and adenosine diphosphate. The latter enzyme cannot form polymers and hence it is of particular interest to ascertain whether a clue to its normal function can be obtained by careful examination of its properties. For example, is this enzyme normally concerned with protein synthesis, oxidative phosphorylation, nucleic acid synthesis or some other process?

Part A cont'd.

(3) In conjunction with the experiments described above under (2), the effect of dialysis on the glycine reductase system of C. sticklandii was reexamined. It appears that enzyme preparations which as a result of dialysis no longer can catalyze the reduction of glycine to acetate and the concommitant synthesis of ATP, can be reactivated by a soluble heat-stable cofactor obtained from partically purified enzyme fractions. The properties of this substance(s) are currently under investigation. Earlier more indirect studies have implicated some type of a quinone catalyst in the overall enzymic reaction.

Proposed of Action:

(1) Further studies on the properties of the oxygenlabile compound necessary for Co_2 reduction to methane.

Investigation of the dinitrophenol sensitive Co_2 fixation reaction into pyruvate as a means of learning something about the mechanism of DNP inhibition in soluble systems.

(2) Purification of the enzyme in C. sticklandii that catalyzes the exchange of PO_4 and ADP in order to learn something about its biological function.

(3) Isolation and characterization of the heat-stable factor(s) necessary for the glycine reductase system.

Part B Included NO

Serial No. NHI-130 (c)

1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effects of MER-29 on Cholesterol Biosynthesis and on Blood Sterols in Man.

Principal Investigators: Daniel Steinberg, Joel Avigan, and Eugene B. Feigelson.

Other Investigators: Hugh E. Vroman

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.2
Professional: 0.7
Other: 0.5

Patient Days: 650

Project Description:

Objectives: To study the clinical effectiveness and mechanism of action of the drug MER-29 (Triparanol). To elucidate the effects of this agent on sterol metabolism in the human organism.

Methods Employed: Patients were maintained on controlled diets and given MER-29 at a dose of 100 or 250 mg daily. Serum desmosterol and cholesterol concentrations were determined during control and drug periods by a colorimetric method developed for this purpose. Radioisotope studies were carried out after injecting intravenously the patients with 2-C^{14}- mevalonic acid. Both the free and esterified sterols were isolated from serum samples taken at various times after administration of label and their specific radioactivities were determined. In some cases a chromatographic fractionation of the sterols was carried out and the specific radioactivities of the isolated desmosterol and cholesterol were determined. Some gas chromatographic analyses of the serum sterols were also carried out.

Patient Material: See above.

Major Findings: Effect on Serum Sterols. As in previous animal studies, all patients treated with MER-29 accumulated desmosterol in the serum at a concentration range of 19 - 43 per cent (average 27%) of total sterols. The free:total ratios for desmosterol and

Major Findings (continued)

for cholesterol were not significantly different from each other. The concentration of serum cholesterol dropped in all patients as a result of the treatment with the drug (average drop 34%), but this was partially compensated by a simultaneous accumulation of desmosterol and the total sterol levels were, on the average, lower by 15% than control values. In one patient with familial hypercholesterolemia, there was actually a slight rise in total serum sterols in response to MER-29. It was demonstrated that some of the usual clinical procedures for determination of serum cholesterol yield low results for total serum sterols in the presence of desmosterol and that for this reason, the response to the drug is erroneously exaggerated when the standard Liebermann-Burchard reaction is used.

Analytical Method: A colorimetric method for determination of desmosterol and cholesterol in a mixture of the two was developed. This method is based on different light absorption spectra of the products of the Liebermann-Burchard reaction with the two sterols. By measuring light absorption at 635 mμ and at 420 mμ and reading optical densities obtained with pure standards, the concentrations of both sterols can be calculated.

Radioisotope Studies: After injection of 2-C^{14}-mevalonate, the peak specific radioactivity and total radioactivity in the unfractionated serum sterols were on the average lower during treatment than during control periods (by 16 per cent and 22 per cent, respectively). The rates of esterification of the two newly synthesized sterols were about equal. The specific radioactivity of serum desmosterol, however, was at least by an order of magnitude higher than that of cholesterol and they did not become equal until two weeks after administration of label. This finding was consistent with a precursor-product relationship of desmosterol and cholesterol. These results also clearly demonstrated that in man, as in animals, the site of the inhibitory action of MER-29 is the reduction of the side-chain of desmosterol, which is the last step in cholesterol biosynthesis.

Significance to Heart Research: MER-29 is the first effective pharmacologic inhibitor of cholesterol biosynthesis applied clinically. The study of the effects of MER-29 on serum sterols is of importance not only as an evaluation of this drug as a useful cholesterol depressing agent, but also by offering a more general type of evidence on the function of specific inhibitors of cholesterol biosynthesis in the control of hypercholesterolemia and atherosclerosis. The finding of desmosterol in patients receiving MER-29 raises the important question of its possible physiological effects and atherogenicity. No direct evidence is available on this question at this time, however, the authors feel that in the absence of such evidence desmosterol should be considered as equally atherogenic to cholesterol. Consequently, in evaluation of the drug MER-29, its effect on the

Significance to Heart Research (continued)

concentration of total serum sterols, and not of cholesterol only, should be considered. By an analytical procedure reported here, it is possible to measure conveniently the levels of both sterols. In contrast to several colorimetric methods widely used in clinical chemistry, this method does not yield low results for total serum sterols in presence of desmosterol. The investigation has clearly demonstrated that in several previous reports the effect of the drug on serum sterols has been exaggerated because of insufficient knowledge of the sterol components and their properties.

Proposed Course of Project: Investigation of changes in cholesterol metabolism under the influence of MER-29 and possibly other pharmacologic agents will be continued. Changes in composition of body sterols and the possible appearance of unusual components will be studied with the aid of gas liquid chromatography. A study aiming to elucidate the metabolic products of desmosterol is also in progress.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Steinberg, D., Avigan, J., and Feigelson, E. B. The identification of 24-dehydrocholesterol in the serum of patients treated with MER-29. Progress in Card. Dis. 2, 586-92 (1960).

Steinberg, D., and Avigan, J. Inhibitors of Cholesterol Biosynthesis with special reference to the mechanism of action of MER-29. Proceedings of Symposium on "Drugs affecting lipid metabolism", Milan, Italy, June 1960. Elsevier Publishing Co. In Press.

Steinberg, D., Avigan, J., and Feigelson, E. B. Effect of MER-29 on cholesterol biosynthesis and on blood sterol levels in man. In preparation.

Honors and Awards relating to this project: None.

Serial No. NHI-131 (c)
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Mechanism of Action of Dietary Fats in Relation to Serum Lipoproteins.

Principal Investigators: Daniel Steinberg, Joel Avigan and Sven Lindstedt.

Other Investigator: Hugh Vroman

Cooperating Unit: Biochemical Division, Karolinska Institute, Stockholm, Sweden (Dr. Sven Lindstedt).

Man Years (calendar year 1960)
Total: 0.9
Professional: 0.3
Other: 0.6

Patient Days (calendar year 1960)
350

Project Description:

Objectives: To determine the nature of the changes in lipoprotein and cholesterol metabolism effected by dietary fats and to explore the mechanisms of action involved.

Methods Employed, Patient Material, and Major Findings: Studies of cholesterol degradation based on the amounts of fecal steroid and bile acid radioactivity excreted after intravenous injection of 4-C^{14} cholesterol failed to demonstrate a significant correlation with the nature of the dietary fat. However, the variability in results between successive fecal collections made it impossible to be certain that small effects were not being masked.

Studies of the turnover of C^{14} cholic acid have been extended to include now approximately 10 patients. Preliminary data suggest a difference in turnover depending on the kind of dietary fat, turnover being faster on corn oil than on coconut oil. Final data should be available this Spring (final purification carried out by Dr. Sven Lindstedt at the Karolinska Institute in Stockholm).

Significance to Heart Research: Since the use of unsaturated fats in the diet offers great promise as an approach to hypercholesterolemia, an understanding of the mechanisms involved is of obvious importance.

Proposed Course of Project: Further bile acid turnover studies are planned for comparison of normals with hypercholesterolemics, and also for comparison of different types of hypercholesterolemics.

Part B included: No.

Serial No. NHI-132
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Biosynthesis of Serum Lipoproteins.

Principal Investigators: Daniel Steinberg and Charles M. Radding

Other Investigators: Philip Eaton

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.7
Professional: 0.3
Other: 0.4

Project Description:

Objectives: To determine the site of synthesis of the serum lipoproteins and to study the factors regulating synthesis.

Methods Employed and Major Findings: Techniques of liver slice incubation and lipoprotein isolation have been previously described (NHI Project Report No. 142, 1959). This year the influence of fatty acid levels on rates of fatty acid oxidation and deposition as triglyceride were studied using 1-C^{14}-palmitate in the medium at different levels. It was shown that the rate of triglyceride synthesis increases over 8-fold as the concentration of free fatty acid in the medium rises from 0.12 to 1.64 μEq/ml. Fatty acid oxidation increases at a similar rate. Epinephrine added in vitro did not increase fatty acid incorporation into glycerides or conversion to CO_2. Glucose increased rates of glyceride synthesis but had little effect on oxidation of fatty acid to CO_2.

Previous studies demonstrated that the rate of production of the protein moiety of lipoproteins was not responsive to changes in the rate of cholesterol synthesis. Yet serum lipid levels (and lipoprotein protein levels) do under some circumstances reflect rates of hepatic synthesis. The present studies were suggested by observations linking rises in serum lipid levels with increased rates of deposition of free fatty acids in the liver. The results are consistent with the hypothesis that fatty liver can be explained

Methods Employed and Major Findings (continued)

on the basis of rapid mobilization of free fatty acids from the periphery to the liver. The next step is to demonstrate directly an increased production of lipoproteins in the presence of high serum fatty acid levels or in livers previously made fatty by norepinephrine injection into the donor animal.

Significance to Heart Research: By studying each of the component pathways of biosynthesis that converge on the production and secretion of lipoproteins it may be possible to develop a more coherent picture of the mechanisms controlling blood lipid levels.

Proposed Course of Research: Further studies of variables controlling lipid synthesis and lipoprotein production and secretion in perfused liver or liver slices.

Part B included: Yes.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Radding, C. M., and Steinberg, D. Studies on the Synthesis and Secretion of Serum Lipoproteins by Rat Liver Slices. J. Clin. Invest. 39: 1560-1569 (1960).

Honors and Awards relating to this project: None.

Serial No. NHI-133

1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of cholesterol biosynthesis and of the effects of inhibitors on sterol metabolism in animal tissues.

Principal Investigators: Joel Avigan and Daniel Steinberg.

Other Investigators: Hugh E. Vroman

Cooperating Units: Section on Steroids, Laboratory of Chemistry, National Institute of Arthritis and Metabolic Diseases.

Man-Years (calendar year 1960)

Total:	1.1
Professional:	0.6
Other:	0.5

Project Description:

Objectives: To study the effects and mechanism of action of MER-29 and of other specific inhibitors of cholesterol biosynthesis and to isolate and characterize sterols acting as metabolic intermediates, with the purpose of elucidating the pathway of cholesterol formation.

Methods Employed: Rats were fed with MER-29 - a cholesterol depressing agent and an inhibitor of cholesterol biosynthesis. Sterols were isolated from the livers and other tissues of these animals. The two main sterol components, desmosterol and cholesterol, were separated by column chromatography of their derivatives. The identity of desmosterol was demonstrated by infrared spectroscopy and other methods. The desmosterol and cholesterol were determined colorimetrically without preceding fractionation by a method developed for this purpose. In the radioactive isotope studies, rats fed with MER-29 were injected with 1-C^{14} acetate and their liver sterols isolated, fractionated and their specific radioactivities determined. Labeled desmosterol was prepared biosynthetically and it was used in in vitro and in vivo studies on conversion of desmosterol to cholesterol. Control and drug fed rats were injected intraportally with a suspension of labeled desmosterol and its conversion to cholesterol

Methods Employed (continued)

was studied. Liver homogenates were incubated with labeled desmosterol and the inhibitory action of MER-29 either fed, or added in vitro, on the formation of labeled cholesterol was determined.

Patient Material: None.

Major Findings: Identification of desmosterol in the tissues of MER-29 fed rats. The non-cholesterol sterol accumulating in the tissues of MER-29 fed rats has been separated from cholesterol by column chromatography of p-phenylazobenzoyl esters and by gas-liquid chromatography of the free sterols and positively identified as desmosterol (24-dehydrocholesterol) by infrared spectroscopy and by other physical and chemical methods.

The Effect of MER-29 on Composition of Tissue Sterols: The proportion of desmosterol in total sterols in tissues of rats fed MER-29 was 79% in serum, 52% in liver, 27% in brain, 36% in muscle and in the range of 44% - 64% in other organs analyzed. MER-29 caused an appreciable reduction in the level of total rat serum sterols, but the effect on total sterol concentrations in other tissues was generally very small.

The Metabolic Role of Desmosterol: The role of desmosterol as a precursor of cholesterol has been demonstrated in the rat treated with MER-29. The conclusion is based on (a) higher specific radioactivity of desmosterol than that of cholesterol when the two are isolated from a liver of a MER-29 fed rat, (b) rapid conversion in vivo of labeled desmosterol into cholesterol, (c) conversion in vitro by rat liver homogenate. Feeding MER-29 inhibited both the in vivo and the in vitro conversion, so did the addition of the drug to the liver homogenate. MER-29 appears to inhibit the reduction of 24, 25 double bond of desmosterol.

Significance to Heart Research: Because of the increasing use of MER-29 and the present trend to apply inhibitors of cholesterol synthesis in management of hypercholesterolemia and atherosclerosis, the elucidation of the mechanism of action of MER-29 is of considerable importance. The studies have also yielded valuable information on the pathway of cholesterol biosynthesis.

Proposed Course of Project: In view of the still incomplete knowledge on the biological mechanism for conversion of lanosterol to cholesterol, it is of interest to study further the various sterol intermediates preceding cholesterol on the synthetic pathway. MER-29 and possibly other inhibitors will be used in order to isolate specific steps of this process and elucidate its mechanism.

Part B included: Yes.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Avigan, J., Steinberg, D., Thompson, M. J., and Mosettig, E. Mechanism of action of MER-29, an inhibitor of cholesterol biosynthesis. Biochem. Biophys. Res. Com. 2, 63-5 (1960).

Avigan, J., Steinberg, D., Thompson, M. J., and Mosettig, E. The mechanism of action of MER-29. Progress in Card. Dis. 2, 525-30 (1960).

Avigan, J., Steinberg, D., Vroman, H. E., Thompson, M. J., and Mosettig, E. Studies of cholesterol biosynthesis. I. The identification of desmosterol in serum and tissues of animals and man treated with MER-29. J. Biol. Chem. 235, 3123 (1960).

Steinberg, D. and Avigan, J. Studies of cholesterol biosynthesis. II. The role of desmosterol in the biosynthesis of cholesterol. J. Biol. Chem. 235, 3127 (1960).

Honors and Awards relating to this project: None

Serial No. NHI-134
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Epinephrine Induced Hyperlipidemia and Fatty Liver.

Principal Investigator: Eugene B. Feigelson

Other Investigators: Daniel Steinberg, William Pfaff

Cooperating Units: Clinic of Surgery, NHI (Dr. Pfaff)

Man Years (calendar year 1960)
Total: 1.3
Professional: 1.0
Other: 0.3

Project Description:

Objectives, Methods Employed, Major Findings: Previous studies have shown that epinephrine can induce elevations of blood cholesterol and blood phospholipids in dogs and in rats. The response is dependent upon the presence of adrenocortical activity. The fact that the serum free fatty acid response to epinephrine is likewise dependent upon adrenocortical activity suggested that the prime effect might be upon mobilization of free fatty acids (the first response in point of time) and that the subsequent rise in serum lipoproteins might be due to deposit of these free fatty acids in the liver.

Dogs under pentobarbital anesthesia were given intravenous infusions of epinephrine or norepinephrine and open liver biopsies were taken at zero time and at intervals up to 8 hours. Serum free fatty acid levels rose to about 2 μEq/ml. and were maintained there with norepinephrine. The triglyceride content of the liver rose progressively during norepinephrine infusion, reaching levels up to tenfold the initial level. That this was not due to a direct effect of the norepinephrine on liver metabolism was shown by the failure to induce fatty liver by intraportal infusion of norepinephrine at the same rate (1 μgm/kg/min). Histologic specimens showed diffuse deposition of lipid stained with Oil Red O throughout the parenchyma. In some, but not in all, animals, the increase in liver neutral fat was accompanied by increases in serum cholesterol levels but no increase in triglyceride levels.

330

Objectives, Methods Employed, Major Findings (continued)

Analysis of the fatty acids in the triglyceride fraction of the liver showed that there was no decrease in linoleic acid content. Since the liver cannot synthesize this acid, the source of the new lipid is most likely the free fatty acids mobilized from adipose tissue depots.

Significance to Heart Research: From these studies has emerged a working hypothesis regarding hypercholesterolemia of stress: over-activity of the adrenal medulla and cortex elevates circulating levels of catechol amines and corticosteroids. The latter potentiates the lipid-mobilizing action of the former. Excessive amounts of free fatty acids are mobilized, transported to the liver where they are deposited at a rate in excess of the ability of the liver to oxidize them. This results in accumulation of triglycerides which in turn, by a mechanism not yet understood, leads to increased production of lipoproteins with a resultant rise in serum cholesterol and phospholipid levels. (Parallel studies with isolated liver slices (Eaton and Steinberg) have shown that the rate of triglyceride synthesis increases with the level of free fatty acids in the medium.)

In addition, these studies permit inferences to be drawn regarding the mechanism of fatty liver production in general. Here a fatty liver is induced in the presence of normal hepatic function, on the basis of excessive rates of mobilization of free fatty acids. A damaged liver may well be unable to metabolize fully fatty acids brought to it even at a normal rate. In the former case, part of the excess lipid reappears in the serum with consequent elevation of serum lipoprotein levels; in the latter case the ability of the liver to synthesize and secrete lipoproteins may be reduced and serum lipid levels would be normal or low. Hybrid combinations of the two may also be visualized.

Proposed Course of Project: Further slice experiments are contemplated in an attempt to directly demonstrate the proposed elevated rates of lipoprotein production in the fat-laden liver.

Part B included: Yes.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Shafrir, E. and Steinberg, D. The Essential Role of the Adrenal Cortex in the Response of Plasma Free Fatty Acids, Cholesterol, and Phospholipids to Epinephrine Injection. J. Clin. Invest. 39: 310-319 (1960).

Shafrir, E., Sussman, K. E., and Steinberg, D. Role of the Pituitary and the Adrenal in the Mobilization of Free Fatty Acids and Lipoproteins. J. Lipid Research. In Press.

Honors and Awards relating to this project: None.

Serial No. NHI-135

1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Synthesis of alpha-glycerophosphate in adipose tissue.

Principal Investigator: Simeon Margolis

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: To study the synthesis and utilization of alpha-glycerophosphate (αGP) for neutral lipid synthesis in cell-free preparations of rat adipose tissue.

Methods Employed: Substitution of other substrates for the αGP in a system synthesizing glycerides gave an estimate of αGP formation. αGP synthesis was also measured directly by an enzyme assay method. Lipid fractionation with solvents and column chromatography, radioassay of carbon ^{14}, and paper chromatography were employed.

Patient Material: None

Major Findings: αGP can be synthesized from several of the phosphorylated intermediates of the glycolytic pathway when DPNH is added to the system. Glucose, glycogen and glycerol did not serve as precursors of αGP.

Significance to Heart Research: This is part of the basic research program of the Heart Institute.

Proposed Course of Project: Further studies are planned to investigate the role of hormones in the control of αGP synthesis.

Part B Included: No.

Serial No. NHI-136
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Development of a Method for Counting Water Soluble Compounds in the Liquid Scintillation Counter. (Started April, 1958 - Completed)

Principal Investigator: Daniel Steinberg

Other Investigator: None

Cooperating Units: None

This project was completed in 1959.

Part B included: Yes.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Steinberg, D. A New Approach to Radioassay of Aqueous Solutions in the Liquid Scintillation Spectrometer. Analytical Biochem. 1: 23-39 (1960).

Honors and Awards relating to this project: None.

Serial No. NHI-137
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Synthesis and Degradation of Triglycerides in Adipose Tissue Homogenates.

Principal Investigators: D. Steinberg, M. Vaughan, S. Margolis, P. Goldman.

Other Investigators: H. Price and R. Pittman

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.8
Professional: 1.1
Other: 0.7

Project Description:

Objectives: To study the mechanisms of synthesis and degradation of triglycerides in adipose tissue and to attempt to relate hormonal effects on fatty acid release to these mechanisms.

Methods Employed: In vitro incubation of cell free extracts and subcellular fractions from adipose tissue, with measurement of fatty acid release, and incorporation of various radioactive substrates into glycerides.

Separation of radioactive lipids using silicic acid column chromatography, silicic acid paper chromatography, gas liquid chromatography and solvent partition.

Major Findings: The synthesis of triglycerides from α-glycerophosphate and fatty acids by homogenates of adipose tissue has been studied in some detail. The pathway seems similar in general to that described in liver by other workers. The nature of the products synthesized, the effects of varying the concentration of several of the components (including fatty acid which is inhibitory in higher concentration), the time course of changes in glyceride C^{14}, fatty acid concentration and fatty acid specific activity have been described. It has been found that when the homogenate is incubated in vitro lipolysis goes on even when, in the presence of

Major Findings (continued)
suitable cofactors, there is net esterification of fatty acids. The lipase that is active in this system does not have the properties of lipoprotein lipase described by Korn.

More detailed studies of certain aspects of triglyceride synthesis in adipose tissue homogenates are described in separate reports by Doctors Margolis and Goldman.

Significance to Heart Research: An understanding of the intimate mechanisms controlling fatty acid mobilization, in addition to the intrinsic value to basic science, may be helpful in elucidating homeostatic factors involved in the control of blood cholesterol levels.

Proposed Course of Project: Some studies of lipolysis in homogenates are continuing, as are attempts to demonstrate effects of hormones on glyceride synthesis in the homogenate system.

Part B included: No.

Serial No. NHI-138
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Incorporation of C^{14}-labeled Fatty Acids into Glyceride by Adipose Tissue.

Principal Investigators: M. Vaughan, D. Steinberg, D. Deykin

Other Investigators: H. Price and R. Pittman

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.7
Professional: 1.1
Other: 0.6

Project Description:

Objectives: To determine whether the rate of esterification of a radicactive fatty acid is altered concomitant with changes in the rate of fatty acid release produced by hormones and other substances.

Methods Employed: In vitro incubation of adipose tissue with C^{14}-labeled fatty acid; isolation of and determination of radioactivity in glycerides and free fatty acids (FFA) from tissues.

Major Findings: Since both triglyceride synthesis and breakdown appear to go on constantly and simultaneously in adipose tissue, increased amounts of FFA could be accumulated either by increasing the rate of lipolysis or by decreasing the rate of esterification. The incorporation of C^{14}-palmitate into tissue glycerides was increased by the addition of glucose to the medium (reported also by others), and was decreased by the addition of epinephrine, ACTH or glucagon. These effects correlate (qualitatively, not quantitatively) in a negative fashion with simultaneously determined changes in FFA release. Serotonin, which did not alter FFA release, did not affect the incorporation of palmitate into glycerides. In adipose tissue from rats treated with thiouracil the effect of epinephrine on the esterification of C^{14}-palmitic acid is much smaller than it is in tissues from normal rats, as is the effect of epinephrine on FFA release (the latter reported by others and confirmed by us).

Major Findings (continued)

In these experiments the specific activity of the tissue fatty acids was determined at the end of the incubation period. If it is assumed that this is the pool from which triglyceride is synthesized, then it is possible to calculate the effects of various conditions and materials on the rate of esterification of fatty acids. The final interpretation of these studies, however, awaits the results of experiments which are in progress concerning the nature of the pool or pools of FFA within the tissue.

Significance to Heart Research: An understanding of the mechanisms controlling fatty acid mobilization may be helpful in elucidating the factors involved in the control of blood cholesterol.

Proposed Course of Project: Current studies relate to (1) the nature of the free fatty acid in adipose tissue, its localization in the cell, the nature of the pool of fatty acid precursor for glyceride synthesis, and (2) the possibility that changes in the rate of triglyceride breakdown in tissue occur along with changes in rates of synthesis (presuming that such can be proven) and account for the lack of quantitative correlation between alterations in rate of triglyceride synthesis and in rate of fatty acid release.

Part B included: Yes.

PHS-NIH
Individual Project Report
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Steinberg, D., Vaughan, M., and Margolis, S. Control of fatty acid release from adipose tissue through control of the rate of triglyceride synthesis. J. Biol. Chem. 235: PC 38 (1960).

Honors and Awards relating to this project: None

Serial No. NHI-139
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effects of Hormones on the Metabolism of Adipose Tissue Studied in Vitro
(Other experiments relating to this problem are related in two other Progress Reports)

Principal Investigator: Martha Vaughan

Other Investigators: Ray Pittman, Helen Price

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.8
Professional: 0.4
Other: 0.4

Project Description:

Objectives: To investigate the metabolism of adipose tissue in order to elucidate the mechanism by which this tissue regulates triglyceride synthesis and breakdown providing for storage of energy and its release in the form of fatty acids. To study the mechanism of action of the several hormones which apparently participate in the regulation of its metabolism.

Methods Employed: In vitro incubation of whole adipose tissue and broken cell preparations with measurements of the amounts of several carbohydrate and lipid metabolites. Radioassay of C^{14} in various metabolic intermediates.

Major Findings: It was reported last year that epinephrine, ACTH, and glucagon all increase phosphorylase activity, stimulate the release of free fatty acids (FFA) and accelerate glucose uptake when added to adipose tissue incubated in vitro. Serotonin also stimulates glucose uptake and increases phosphorylase activity but does not stimulate FFA release. The effects of these hormones and some other compounds (e.g., caffeine, 5'-AMP, 3',5'-AMP) on the conversion of C^{14} from uniformly labeled glucose to the glycerol and fatty acid moieties of tissue glycerides was investigated. The

Major Findings (continued)

effects of epinephrine were like those of ACTH. Glucagon produced somewhat different changes in the incorporation of glucose C^{14} into glycerides but its effects were not like those of insulin.

Ergocornine inhibited the effects of epinephrine on fatty acid release without significantly altering its effects on the conversion of C^{14} from glucose to glycerides. This is another bit of evidence which, taken along with a few other observations, suggests that the effects on FFA release may be independent of at least some of the other actions of this hormone.

Significance to Heart Research: An understanding of the mechanisms controlling fatty acid mobilization may be helpful in elucidating the factors involved in the control of blood cholesterol.

Proposed Course of Project: Several other compounds, some of them inhibitors of epinephrine effects, others related in chemical structure to epinephrine and to serotonin, are being studied in similar experiments in an attempt to learn more about the regulation of metabolism in adipose tissue.

Part B included: Yes.

PHS-NIH
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Vaughan, M. Effect of Hormones on Phosphorylase Activity in Adipose Tissue. J. Biol. Chem. 235: 3049-3053 (1960).

Vaughan, M. Effect of Hormones on Glucose Metabolism in Adipose Tissue. J. Biol. Chem. In Press.

Honors and Awards relating to this project: None.

Serial No. NHI-140

1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Triglyceride Synthesis from Diglycerides

Principal Investigator: Peter Goldman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: .8
Professional: .8
Other: 0

Project Description:

Objectives: To study the enzymatic specificity of the synthesis of triglycerides in adipose tissue using various synthetic diglycerides and several fatty acyl coenzyme A derivatives. Major interest is in the conditions and requirements for the step Fatty Acyl CoA + diglyceride → triglyceride.

Methods Employed: Fatty acyl coenzyme A derivatives of 1-C^{14} labeled palmitic and 1-C^{14} labeled oleic acid have been synthesized by a modification of previously described organic synthesis. 1,2 diglycerides containing saturated fatty acids have also been synthesized and several diglycerides of unsaturated fatty acids have been obtained from other investigators. Subfractions of broken cell preparations of adipose tissue from the rat and the chicken are used as an enzyme source. Triglycerides are isolated from the incubation mixture and C^{14} count incorporation used as an indicator of synthesis.

Patient Material: None

Major Findings: Rat adipose tissue contains an active thiolase which rapidly destroys palmitoyl coenzyme A and greatly reduces its incorporation into triglyceride. No method has been successful in disassociating this activity from the desired enzymatic activity. However, in chicken adipose tissue synthesis of triglyceride has been observed from the diglyceride and acyl coenzyme A precursors.

Significance to Heart Research: An understanding of lipid biosynthesis is believed to be pertinent to an understanding of the pathogenesis of atherosclerosis.

Proposed Course of Project: It is proposed to purify the enzyme in adipose tissue which is responsible for the synthesis of triglyceride from diglyceride and to study its substrate specificity with regard to synthetic acyl coenzyme A derivatives and synthetic diglycerides.

Part B included: No.

Serial No. NHI-141

1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Lipoprotein Metabolism in Nephrosis
(Current studies started in 1958)

Principal Investigator: James H. Baxter

Other Investigators: Carlos Schultz (technician)

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.5
Professional: 1.0
Other: 0.5

Project Description: Portions of this project have been completed during the year and are described in the publications listed at the end of the report. Remaining portions are described here.

Objectives: To study the effects of certain factors on lipoprotein metabolism and serum lipoprotein levels in nephrosis.

Methods Employed: The following procedures are being studied: (a) repeated plasmapheresis in rabbits, and (b) changes in diet and food intake in nephrotic rats.

Patient Material: None

Major Findings: (a) Studies on plasmapheresis in dogs (described previously) did not afford completely conclusive results because of technical problems and because the dog, even with nephrosis, apparently does not develop severe hyperlipemia. Current studies employ rabbits.

Bleeding (2 per cent of body weight) once or twice daily, with reinfusion of cells, for periods of 1 to 2 weeks, has consistently been accompanied by increases in serum cholesterol, phospholipids, and particularly triglycerides. Smaller increases in lipids occurred with the sham procedure in which only small quantities of plasma were removed, presumably as a result of excitement and epinephrine release. The study is incomplete, but the results

Major Findings (continued)

suggest that removal of albumin (and other proteins including lipoproteins) causes serum lipoproteins to increase.

(b) Effects of continuous 48-hour glucose infusions in patients with nephrosis were discussed previously. Free fatty acids (FFA) were consistently depressed, but serum lipoproteins usually did not change markedly. An unexpected result was a large increase in very low density lipoproteins and serum triglycerides in a few cases. Similar results have been obtained with feeding of carbohydrate at frequent intervals. This study is being extended in nephrotic rats.

A considerable decrease in serum lipids and lactescence occurred in nephrotic rats on stock diet when they were fasted for 24 hours. However, when glucose solution was substituted for stock diet for 24 hours, there was relatively little decrease in serum lipids and lactescence. It appears that depression of FFA by glucose in nephrosis does not result in a decrease in lipoproteins, or, if it does, the effect is counterbalanced by other effects of the glucose. Also, it appears that glucose (at least for 24 hours) is almost as effective as stock diet in maintaining nephrotic hyperlipemia. Incorporation of C^{14}-glucose in FFA and lipoproteins has been studied in a preliminary experiment. Studies also have been made with varied caloric intake and with continuous versus intermittent feeding.

Significance to Heart Research: Nephrosis often leads secondarily to hypertension and cardiovascular disease. Also, knowledge of lipid metabolism is important to heart research because it is thought that faulty lipid metabolism may play a major role in the causation of atherosclerosis.

Proposed Course of Project: Plans for this project have been indicated above. In addition, because it has been suggested that proteinuria (rather than low serum albumin) may be the cause of hyperlipidemia in nephrosis, studies will be made on serum lipids during repeated infusions of serum albumin to the point of producing proteinuria in normal rats. On the basis of our previous studies on albumin infusions in normal and nephrotic rats, it seems unlikely that a rise in lipids will occur.

At present we are working principally on a new project listed below.

New Project: Studies on Binding of Dye 2-(4-hydroxybenzeneazo)-benzoic acid (HBABA) by Serum Albumin of Various Mammalian Species.

Using a modification of Rutstein's albumin method, we have noted that the 490 mμ extinction varies consistently with the species, and there are differences in the effects in other regions of the dye absorption curve. Quantitative studies on binding of the dye

(New Project, continued)

by various albumins by the method of equilibrium dialysis are in progress. An attempt will be made to correlate the progression of binding to new sites, as concentration of free dye is increased, with changes in spectral absorption of the dye-albumin complex. It seems that it might be possible to distinguish the different albumins by this method, and that something might be learned concerning the differences in binding sites on the different albumins.

Part B Included: Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Baxter, J. H., Goodman, H. C., and Havel, R. J. Serum lipid and lipoprotein alterations in nephrosis. J. Clin. Invest. 39, 455 (1960). (Listed in previous report as accepted for publication).

Baxter, J. H., Goodman, H. C., and Allen, J. C. Effects of infusions of serum albumin on serum lipids and lipoproteins in nephrosis. J. Clin. Invest. Accepted for publication in March, 1961.

Allen, J. C., Baxter, J. H., and Goodman, H. C. Effects of dextran, polyvinylpirrolidone, and gamma globulin on the hyperlipidemia of experimental nephrosis. J. Clin. Invest. Accepted for publication in March, 1961.

Honors and Awards relating to this project: None.

Serial No. NHI-142
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Investigations on the structure of fibrinogen.

Principal Investigator: Elemer Mihalyi

Other Investigators: Jamie E. Godfrey

Cooperating Units: None

Man Years: Total 2.0 (calendar year 1960)
Professional: 2.0
Other: 0

Project Description:

Objectives: Study of the chemical and physicochemical structure of fibrinogen with special emphasis on the proteolytic fragmentation of the fibrinogen molecule.

Methods Employed: Amino acid analysis (Spinco automatic amino acid analyzer and chemical methods), pH-stat, viscosity, optical rotation and sedimentation in the ultracentrifuge.

Patient Material: None.

Major Findings: The complete amino acid composition of fibrinogen has been obtained. The protein has been hydrolyzed for varying time intervals and thus the hydrolytic losses of each amino acid determined. The amino acid recoveries extrapolated to zero hydrolysis time have been compared then to the total nitrogen values. This procedure showed essentially complete recovery of the total nitrogen in the amino acids determined. The data derived are necessary to know the exact number of susceptible bonds in the molecule toward various enzymes and chemical reagents. Next the enzymatic breakdown of fibrinogen has been investigated. Most of the work has been performed with trypsin, the reaction being followed in the pH-stat, viscometer, ultracentrifuge and polarimeter. All these data show that fibrinogen, in a rapid reaction, is broken up in several large fragments of practically identical shape and size. This reaction proceeds through the rupture of approximately 70 peptide bonds in the fibrinogen molecule of 350,000 molecular weight. The reaction then proceeds to the

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Major Findings (continued)

rest of the susceptible bonds which are broken much more slowly. In this later phase several classes of bonds can be distinguished according to their rate of splitting. Whereas in the first phase there is only a small percentage of non protein nitrogen liberated (cca 5%) the second, slow phase, results in the gradual degradation of fibrinogen to low molecular weight peptides.

Our attention has been focused mainly on the first phase and the large fragments formed during this phase. The fragment was isolated in good yields and sufficient purity by an ammonium sulphate fractionation. Its molecular weight has been established from sedimentation-diffusion and approach to the sedimentation equilibrium measurements as approximately 100,000. The shape of the fragment is nearly spherical. It may be recalled that in the electronmicroscope (Hall) fibrinogen looks like a formation of three spherical parts united by thin filaments. It appears that trypsin cuts through the filaments liberating thus the spherical substructures.

The fragments do not react with thrombin any longer, what is not surprising in view of the similar specificities of the two enzymes. The clottability of fibrinogen disappears even before the fragments are liberated, thus it appears that some bonds in the essential part of the fibrinogen molecule are split at a much faster rate than those connected with the liberation of the fragments.

Chymotrypsin and plasmin liberate the same fragments from fibrinogen. However, these fragments still contain the bonds susceptible to thrombin. They do not polymerize any longer, but the action of thrombin can be demonstrated in the pH-stat.

Significance to Heart Research: Proteolytic dissolution of intravascular fibrin clots seems to be of some use in reestablishing circulation in coronary thrombosis. Investigation of the proteolytic degradation of fibrinogen may be thus of importance to heart research.

Proposed Course of Project: The work on the digestion of fibrinogen with trypsin and properties of the fragment has been completed and two papers on this subject are in preparation. In the future we plan to investigate the reaction of the fragment obtained with chymotrypsin and plasmin with thrombin. These fragments do not clot, thus make possible the study of the limited proteolysis brought about by thrombin with usual techniques, without the complication caused by gelation.

Part B Included: No.

Serial No. NHI-143 (c)
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of Hyperlipidemic States in Humans (project begun in 1956 - a continuing study).

Principal Investigator: Donald S. Fredrickson

Other Investigators: Mr. Katsuto Ono, technician
Mr. Louis Davis (summer worker), technician

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.6
Professional: 0.2
Other: 0.4

Patient Days (calendar year 1960)
300

Project Description:

Objectives: The continuing study of diseases characterized by "primary" elevations of lipid concentrations in extracellular fluid (Essential Hyperlipidemia). Emphasis is placed upon search for aberrant biochemical mechanisms in these conditions, but study is also directed toward classification, course, genetics, and treatment. Major objectives pursued this year were: (1) development of a method for assaying post-heparin lipolytic activity in plasma in a quantitative and reproducible manner; and to determine if this assay might help segregate various biochemical traits leading to hyperlipemia; (2) further study of pedigrees with hyperlipidemia to determine the breadth and nature of the chemical expression of the abnormal trait.

Methods Employed: A weekly outpatient clinic is maintained at which referred patients are seen and followed. A pool of patients with hyperlipidemia is maintained for studies in this and related projects. Methods for practically complete fractionation and quantification of practically all the plasma lipids and lipoproteins are available.

Experimental drugs and diets are used in both in- and out-patient studies.

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Patient Material: Subjects used for studies this year included: (1) patients with abnormal blood lipids referred to us by outside physicians as well as members of our local "panel" of about 100 hyperlipidemic patients. One large family was studied in connection with the Baltimore Marine Hospital. In many instances samples of blood from relatives of patients are sent to us for analysis. Several large pedigrees have been characterized in this manner. (2) Normal control subjects from the regular Volunteer Program at the National Institutes of Health.

Major Findings: (1) A standardized test for post-heparin lipolytic activity in plasma was developed. This assay involves the measurement of rate of lipolysis under conditions in which there is definite excess of substrate and acceptor for released fatty acids. Despite the obvious features of the test, no such assay has ever been published for clinical work since the clearing phenomenon was reported in 1946. It was necessary to add more substrate than previously thought in order to overcome an undefined inhibitor in plasma. Also evaluated were the effect of added cation, the dose and time response to heparin, presence of inhibitors and stimulators, and the reproducibility and meaning of the test explored. Hundreds of assays in about 75 normal and hyperlipidemic subjects were made this year.

(2) The post-heparin lipolytic response in man is very reproducible, and in most normolipidemic individuals falls closely about a rate value, in this test of: 0.2-0.4 μEq. fatty acid released per minute per ml of plasma (10 minutes after injection of 0.1 mg sodium heparin per kg. body weight).

(3) The response in hyperlipemic subjects is over a much wider range (0.1 to 0.8). There is no definite segregation. However, it is suggested that there are three "categories of response" in hyperlipemics: (1) lower than normal; this is the smallest group, and includes the well-known Presbury family, studied here. These patients may have a true defect in removal of chylomicrons, associated with this lower enzyme response; (2) normal values, includes most of the hyperlipemic members of families having the trait known as Essential Familial Hypercholesterolemia; (3) higher than normal, possibly an adaptive response in some patients to continued hyperlipemia.

(4) The post-heparin response in man is definitely related to the fat intake. Subjects on very low fat diets can have levels of activity lower than patients with severe familial hyperlipemia,

Major Findings (continued)

yet their plasma triglycerides are normal. Dependency of activity upon shifts in diet have been demonstrated in normals.

(5) All the activity was not salt inhibitable. It is entirely possible that the post-heparin lipolytic activity is due to more than one enzyme.

(6) In summary: This test may ultimately prove to have value in segregating hyperlipemic traits. Strict attention to dietary preparation is essential to interpretation. Some patients formerly thought to have familial lack of the enzyme response have been shown to have activity levels in the low normal range. The absence of the activity is not the genetically determined defect per se; it may be failure to develop activity in vivo, of course, since the test only measures the potential for response under optimal conditions in vitro.

Significance to Heart Research: Most of the patients in the category under study have a significantly higher incidence of coronary artery disease presumably related to some abnormal aspects of lipid metabolism. They are encountered frequently by the average physician. Better methods of classification and treatment are urgently needed.

Proposed Course of Project: The general course of the project will be a continuation of the preceding year.

Part B included: Yes.

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Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Fredrickson, D. C. The Meaning of Hyperlipidemia in "Lipids and the Steroid Hormones in Clinical Medicine", ed. F. W. Sunderman and F. W. Sunderman, Jr., Philadelphia, J. B. Lippincott Co., 1960.

Fredrickson, D. S. Phospholipid Separation in "Lipids and the Steroid Hormones in Clinical Medicine", ed. F. W. Sunderman and F. W. Sunderman, Jr., Philadelphia, J. B. Lippincott Co., 1960.

Honors and Awards relating to this project: None.

Serial No. NHI-144 (c)
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Study of Tissue Lipidoses

Principal Investigator: Donald S. Fredrickson

Other Investigators: Dr. Paul Altrocchi (for 6 months, from July, 1960), Dr. Arthur Karmen, Mr. Katsuto Ono, technician.

Cooperating Units: Dr. Robert Krooth, NIAID (Tissue culture).

Man Years (calendar year 1960)		Patient Days (calendar year 1960)
Total:	1.1	530
Professional:	0.8	
Other:	0.3	

Project Description:

Objectives: A continuing study of the rare diseases characterized by lipid storage in tissues, primarily those due to genetically determined defects in lipid metabolism. The aim is to search for the biochemical "lesions" in these diseases, to sharpen our insight into the clinical and genetic behavior of such conditions, and to use this information to enrich our understanding of the basic mechanisms for transporting and metabolizing lipids.

The specific objectives of this year were:

(1) Study of a form of cholesterolosis which may represent a hitherto unreported type of genetically determined lipidosis, and involving a fascinating population isolate on the Island of Tangier in Chesapeake Bay.

(2) Study of white cells as a readily available source material for identification of lipid abnormalities in the reticuloendothelial tissues both for ease of diagnosis and discovery to responsible enzyme defects.

(3) Further observation of a rare case of hypercarotenemia.

(4) Study of the lipid abnormality in Niemann-Pick Disease.

Methods Employed:

(1) Tissues were analyzed from a five year old boy with lipid laden foam cells in many reticuloendothelial (RE) tissues. Both conventional silicic acid and gas liquid chromatography were used. Examination of about 50 residents on the Island of Tangier, Virginia, revealed that the patient's sister also had very strange appearing tonsils. These were removed at the Clinical Center. A second cousin of the patient with benign tonsillar hypertrophy, who desired tonsillectomy, was also brought here to supply control tonsillar tissue for both chemical assay and biochemical studies. Immediately after removal, tonsil slices were incubated with labeled precursors of cholesterol to compare synthetic rates in these tissues. A complete pedigree of these children through six generations was also prepared.

(2) White cells were isolated from venous or arterial blood samples (50-100 ml.), the lipids extracted and chromatographed on silicic acid. Fractions separated included four groups of phospholipids, cholesterol, both free and esterified, and glycerides. These were selectively hydrolyzed and the fatty acid composition determined on gas liquid chromatography.

(3) Radioactive beta carotene was prepared by incubation of labeled carbon dioxide with Chlorella. Methods for separating carotene, vitamin A and retinine were also set up. A diet containing no carotene or vitamin A was fed to the hypercarotenemic child. The plasma levels of both carotene and vitamin A were followed during this time and after addition of large amounts of carotene or vitamin A.

Patient Material: Patients include children and adults referred to us with either a definite or an uncertain diagnosis of lipid storage disease. Frequently, the parents and some other relatives of the patients are admitted for a brief period to permit characterization of the pedigree by one or more clinical tests. As noted, patient material this year was recruited from Tangier Island, Va., in the study of the heretofore undescribed lipid storage disease.

Major Findings:

(1) From results so far, it appears that a new familial lipidosis may have been discovered on the Island of Tangier. It is manifested by the accumulation of foam cells in lymph nodes, liver, spleen, and tonsils. The only lipid elevated is cholesterol which is present mainly as esters. So far, these esters contain fatty acids whose relative composition is different from that in the plasma sterol esters. The only sterol accumulating is cholesterol. These patients have many features of Niemann-Pick disease; the offending lipid is similar to that reported for Hand-Schuller Christian syndrome, but

Major Findings (continued)

none of the typical pathological lesions have been seen. Comparison of the lipid content of tissues in these two diseases is now underway. From the pedigree study, it is apparent that these two involved siblings may represent the homozygous genotype of a rare recessive gene present in a mutual great, great, great-grandfather. The isotopic studies are incomplete. One fascinating aspect of the studies so far is the indication that the tonsil may be a superbly available and useful tissue for the study of lipid synthesis in the RE system.

(2) The white cell studies have so far been limited mainly to establishment of normal data.

(3) The "balance" studies strongly suggest that the hypercarotenemic patient has a defect in conversion of carotene to vitamin A. The evidence for this includes the failure of vitamin A levels in plasma to budge from a low level despite a marked rise in carotene after feeding the latter. Vitamin A is well-absorbed by the patient and plasma disappearance curves have been measured after different periods of leading of Vitamin A. Preparations have been completed for tracer studies of the conversion in this patient. If this defect is present, it will be the first time that such a defect has been demonstrated in man.

Significance to Heart Research: The elucidation of biochemical mechanisms underlying clinically apparent defects in lipid metabolism can be of great importance to understanding some of the aspects of atherosclerosis - a disease vaguely but definitely related to abnormal lipid transport. The patients with "cholesterolosis (Tangier type)" offer an especially promising approach to familial defects causing deposition of cholesterol in tissues.

Proposed Course of Project: The two aspects of this study which will be most intensively investigated in the coming year are the use of the white cells as a reference tissue for the diagnosis of the lipidoses and as a means of investigating the inheritable metabolic defect; and the use of tonsillar tissue to study lipid metabolism in the reticuloendothelial system.

Part B included - Yes.

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S. Inherited Variation and Metabolic Abnormality, in "The Metabolic Basis of Inherited Disease", ed. Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., New York, McGraw-Hill, 1960.

Fredrickson, D. S. Essential Familial Hyperlipidemia, in "The Metabolic Basis of Inherited Disease", ed. Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., New York, McGraw-Hill, 1960.

Fredrickson, D. S. Infantile Amaurotic Family Idiocy, in "The Metabolic Basis of Inherited Disease", ed. Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., New York, McGraw-Hill, 1960.

Fredrickson, D. S. Gaucher's Disease, in "The Metabolic Basis of Inherited Disease", ed. Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., New York, McGraw-Hill, 1960.

Fredrickson, D. S. Niemann-Pick Disease, in "The Metabolic Basis of Inherited Disease", ed. Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., New York, McGraw-Hill, 1960.

Honors and Awards relating to this project: None.

Serial No. NHI-145
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of the mechanisms of fat transport and metabolism.

Principal Investigator: Donald S. Fredrickson

Other Investigators: Collaborating: Dr. John Stephenson (LTD-NHI), Mr. Arnold Jones (LTD-NHI), Mr. Katsuto Ono

Cooperating Units: Dr. Leonard Laster - NIAMD; Dr. Howard C. Goodman - NIAID.

Man Years (Calendar year 1960)
Total: 2.0
Professional: 1.5
Other: 0.5

Patient Days (Calendar year 1960)
None (Patient not on our Service)

Project Description:

Objectives: A study of the plasma transport of fatty acids as free fatty acids (FFA) and as chylomicron triglyceride fatty acids (TGFA). Three specific problems, although closely related, are involved:

(1) The metabolism of isomeric members of some of the normal series of unsaturated fatty acids (Mr. Ono).

(2) Continued kinetic analyses of turnover data previously obtained concerning free fatty acid transport in plasma.

(3) Chylomicron formation and fat transport in a patient with hereditary absence of β-lipoprotein.

Methods Employed and Major Findings:

(1) Limited during this period to development of methods for preparation of C^{14}-labeled trans and cis trans isomers of linoleic and oleic acids.

(2) Mr. Jones has successfully programmed for the computer the probabilistic computations (per Dr. Stephenson's theory) begun last year of the radioactive tracer data previously obtained in

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Methods Employed and Major Findings (continued)

study of plasma FFA metabolism. These computations are now complete and remain to be plotted and collated. These exceedingly tedious analyses have indicated several more experiments to be done early next year. At best, some definite estimates of the importance of FFA transport to overall body metabolism will be obtained; at worst, the limitations of isotopic studies in this area will be specifically demonstrated.

(3) A patient without demonstrable β-lipoprotein has become available to us. The turnover of this lipoprotein is being studied by immunochemical techniques. Since these rare patients do not seem to form chylomicrons these studies should bear directly on the nature and function of chylomicron proteins previously studied here. Studies so far indicate no obvious means for absorbing fat, normal red cell total lipid, and low post-heparin lipoprotein lipase activity. Antigen for antibody to β-lipoprotein has also been prepared.

Patient Material: The above rare case of α-beta-lipoproteinemia is a patient of Dr. Leonard Laster (NIAMD).

Significance to Heart Research: Because of the association of accelerated atherosclerosis and hyperlipidemia, the normal mechanisms of fat transport are one of the most reasonable avenues of approach to a basis of understanding this important cardiovascular disease.

Proposed Course of Project: The metabolic behavior of the cis and trans isomers will be observed. The analyses should be completed in the Spring. The α-beta-lipoproteinemic patient will receive exogenous β-lipoprotein for measurement of turnover and effects on chylomicron formation.

Part B includes: No.

Serial No. NHI-146
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Metabolism of Different Fatty Acids: A Simultaneous Comparison Using Differentially-labeled Tracer Fatty Acids.

Principal Investigators: J. P. Dustin (Visiting Scientist, University of Brussels) and Donald S. Fredrickson.

Other Investigators: Phillipe Laudat and Katsuto Ono

Cooperating Units: Dr. Arthur Karmen (LTD-NHI) gas chromatography

Man Years (calendar year 1960):

Total:	1.5
Professional:	1.2
Other:	0.3

Project Description:

Objectives: The comparison of the transport in plasma and the utilization in tissues of two important fatty acids differing significantly in structure and possibly metabolic function.

Methods Employed: Since the comparison of the metabolism of two long chain fatty acids must be as independent as possible of undetermined variables, the simultaneous observation of the metabolism of the two fatty acids was considered essential. For this purpose intensive study was made of simultaneous counting of carbon and tritium in the liquid scintillation spectrometer, and a suitable method devised. Carbon-labeled linoleic acid and tritiated palmitic acid were then used simultaneously to: (1) measure rates of disappearance of the acids when injected as albumin-bound free fatty acids (FFA); (2) appearance in the chylomicrons when fed; and (3) behavior of the acids as triglyceride fatty acid (TGFA). Measurements were made in both plasma lipids and in chromatographically separated lipids in liver samples removed at intervals.

Major Findings: Lengthy experiments involving five dogs have been completed but not all of the data analyzed. So far, an interesting pattern of removal of FFA from the blood is observable. Superficially the curves for linoleic and palmitic acids appear the same, and we have previously reported that they are identical during the early phase of removal. When the isotopic ratios are measured, this much more sensitive index of comparative rates of removal indicates that labeled linoleic acid is leaving the plasma much faster than labeled palmitic acid. A possible explanation for this is found in the liver lecithin fraction, in which as much as one-third of the labeled linoleic acid may be present four minutes after injection. The appearance of linoleic in the liver phospholipid fractions is much more rapid than palmitic. No other liver lipid accounts for so much early uptake of labeled FFA from the plasma, hence the relative rates of exchange of labeled acids with the liver lecithins may be the major determinant of the pattern of FFA removal from the plasma. On the other hand, these two labeled acids, when removed from plasma as triglyceride, leave at identical rates and the participation of the liver lecithin pool is much less. Further study is obviously in order, but the technique shows considerable promise in eliminating spontaneous variation in comparison of the metabolism of two closely related chemical species.

Proposed Course of Project: Immediate problems are further analysis of the data, determination of chemical composition of plasma and tissue fractions in which radioactivity is being measured, and determination of the positional labeling in the triglycerides obtained after feeding and injected into recipients.

Significance to Heart Research: The comparative metabolism of various fatty acids is currently of interest in regard to many aspects of fat metabolism, some of which are particularly related to the problem of atherogenesis.

Part B included: No.

Serial No. NHI-147

1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Metabolism of Free Fatty Acids (FFA)

Principal Investigator: Robert S. Gordon, Jr.

Other Investigators: Dr. Hans H. Ott (Visiting Fellow)

Cooperating Units: Laboratory of Technical Development, NHI (Dr. Hayes)

Man Years (calendar year 1960)
Total: .75
Professional: .75
Other: 0

Project Description:

Objectives: The principal objective is further to elucidate the means whereby FFA are liberated from adipose tissue, transported in the blood, and utilized.

Methods Employed: Previous physiologic studies were not continued during calendar year 1960 but instead investigations concerning the physical chemistry of the formation of albumin fatty acid complexes were carried out. For this study a stop-flow apparatus attached to the Beckman spectrophotometer was employed. In addition, preliminary experiments involving the use of the tri-carb scintillation counter for following the diffusion of fatty acids were run.

Patient Material: None

Major Findings: Studies carried out primarily by Drs. Hans Ott and Joseph E. Hayes concern measurements of the kinetics of the formation of complexes between human serum albumin and FFA. When these complexes form in a solution buffered at a pH close to 11 there is an appreciable change in the ultraviolet absorption due to albumin. It was felt that following the change of UV absorption would make it possible to compute the rate of complex formation. Measurements were carried out in a stop-flow apparatus of the type used by Chance. The formation of albumin fatty acid complex proved to be too rapid for accurate measurement by this technique. These results are being readied for publication.

Major Findings (continued)

In another study, attempts were made to demonstrate the diffusion of FFA ions through gel media with and without serum albumin. The diffusion of the fatty acid, which was labeled with C^{14}, was evidenced by counts arising in a plastic fluor placed adjacent to the gel. These counts were recorded by the automatic sample changer of the Packard tri-carb scintillation counter. These preliminary experiments were not quantitative but suggested a means whereby the question could be investigated carefully. The data available showed that free fatty ions in free solution diffused much more rapidly than does albumin itself, as might have been expected. The rate at which fatty acid diffuses through an albumin-containing medium is not appreciably greater than the diffusion rate of the albumin itself.

Significance to Heart Research: FFA's have been shown to be important metabolites in many tissues including myocardium. Because of their relationship to other serum lipids and possibly to atherogenesis, a continued study of their metabolism is felt to be important.

Proposed Course of Project: It is hoped that these studies can be continued at some time in the future when a Visiting Fellow or other member of the laboratory is available to spend more time on them. No work in connection with these projects is underway at this time.

Part B included: Yes.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Gordon, R. S., Jr. Free Fatty Acids in Serum A. Analytical Considerations and Clinical Significance. In "Lipids and the Steroid Hormones in Clinical Medicine", ed. F. W. Sunderman and F. W. Sunderman, Jr., Philadelphia, J. B. Lippincott Co., 1960.

Gordon, R. S., Jr. Free Fatty Acids in Serum B. Method for Free Fatty Acids in Serum. In "Lipids and the Steroid Hormones in Clinical Medicine", ed. F. W. Sunderman and F. W. Sunderman, Jr., Philadelphia, J. B. Lippincott Co., 1960.

Honors and Awards relating to this project: None.

Serial No. NHI-148 (c)
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Fecal Fatty Acids.

Principal Investigator: Robert S. Gordon, Jr.

Other Investigators: William C. Watson

Cooperating Units: Laboratory of Technical Development, NHI (Dr. Arthur Karmen)

Man Years (calendar year 1960):
Total: .5
Professional: .5
Other: 0

Patient Days (calendar year 1960)
None. (This study has utilized patient material only incidentally; no patient has been admitted on account of this study)

Project Description:

Objectives: To learn more about the composition of fecal fats and in particular to investigate the absorption and mechanism of action of castor oil.

Methods Employed: Fecal fatty acids have been determined by titration, by oxidation with chromic acid and by gas chromatography. Procedures for carrying out these analyses have been applied to normals, patients with steatorrhea and patients receiving castor oil in preparation for x-ray procedures. Castor oil-containing diets have been fed to rats and the feces, thoracic duct lymph, and body fat have been analyzed.

Patient Material: As above.

Major Findings: The absorption of ricinoleic acid in rats has been conclusively proved by its identification in thoracic duct lymph and in body fat. The amount fed is not recovered quantitatively in the feces of humans but the interpretation on this finding is somewhat difficult. Normal human feces have been found to contain a variety of fatty acids with a marked preponderance of the saturated ones (chiefly stearic, some palmitic). The observation of James (Biochem. J., Feb. 1960), that some feces contain hydroxylated fatty acids has

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Major Findings (continued)

been confirmed. The presence of such hydroxy acids in feces of patients not receiving castor oil renders the interpretation of balance studies with ricinoleic acid difficult.

Significance to Heart Research: A study of fecal fatty acids and of the absorption and mechanism of action of ricinoleic acid may contribute further to understanding of basic problems related to fat absorption. The relationship of dietary fat to serum cholesterol levels is well known.

Proposed Course of Project: Further experiments will be undertaken along the lines already indicated.

Part B included: No.

Serial No. NHI-149(c)
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Clinical Studies on Plasma Protein Metabolism

Principal Investigator: Robert S. Gordon, Jr.

Other Investigators: Dr. Hans H. Ott (Guest Worker)

Cooperating Units: Dr. Thomas Waldmann, Metabolism Section, NCI

Man Years (Calendar year 1960)
Total: .75
Professional: .75
Other: 0.4

Patient Days (Calendar year 1960)
500

Project Description:

Objectives: The principal objective of this study has been to discover the disturbances of physiology responsible for the clinical syndromes formerly called "idiopathic hypoproteinemia".

Methods Employed and Patient Material: Patients with clinical problems illustrative of various aspects of hypoproteinemia have been admitted to the Clinical Center and, where necessary, normal controls have been utilized for comparison. In addition to the usual clinical tests these patients have been investigated by the use of I^{131}-polyvinylpyrrolidone (PVP) and I^{131}-albumin, and the morphology of their digestive tract has been studied with the aid of suction biopsies.

Major Findings: More has been learned about the clinical conditions of which hypercatabolic hypoproteinemia is a manifestation. Some patients presenting with this syndrome have been found to have well-known gastrointestinal diseases which had not been recognized in these individual cases. Among such patients are examples of nontropical sprue, giant rugal hypertrophy of the stomach and regional enteritis. Another case in which gastrointestinal protein loss accompanied constrictive pericarditis was uncovered. By far the largest number of cases, however, has been found to be attributable to a disorder of gastrointestinal lymphatic vessels which had not previously been recognized. Twelve cases of this type have been studied and histologic material has become available. A second form

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Major Findings (continued)

of hypoalbuminemia, that attributable to congenital failure of albumin production, is known and a second patient with this disease has been studied at the Clinical Center. This patient and the one case of analbuminemia previously known at this Institute were given S^{35}-labeled yeast protein hydrolysate by mouth and the amount of radioactivity in plasma protein fractions was followed for several weeks. Each individual showed slight but definite incorporation of radioactivity into serum albumin.

Finally, throughout 1960 the production of I^{131} has been continued with the help of the Clinical Center Pharmacy Department. This test substance has been made available to qualified investigators all over the world to permit the documentation of other cases of "protein losing gastroenteropathy". Help has also been offered to American companies interested in the commercial production of I^{131} PVP.

Significance to Heart Research: Investigation of hypoalbuminemia is of indirect importance to heart research, inasmuch as it will increase understanding of mechanisms of edema formation. Subsequent loss of plasma protein into gastrointestinal tract has been discovered in three cases of constrictive pericarditis and in one patient suffering from interatrial septal defect. It is possible that this process is of significance in the hypoproteinemia associated with certain cardiac conditions (especially constrictive pericarditis).

Proposed Course of Project: It is hoped that more patients with hypoproteinemias of various causes can be admitted to the Clinical Center and studied. This will provide a broader base for generalizations concerning the nature and course of the diseases involved. Follow-up will be maintained wherever possible on the patients who have been discharged in connection with this study.

Part B included: Yes.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Gordon, Robert S., Jr. Hereditary Hypoproteinemias and Other Plasma Protein Abnormalities. Chapter in "The Metabolic Basis of Inherited Disease", edited by Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., New York, McGraw-Hill, 1960.

Steinfeld, J. L., Davidson, J. D., Gordon, R. S., Jr., and Greene, F. E. The Mechanism of Hypoproteinemia in Patients with Regional Enteritis and Ulcerative Colitis. The Amer. J. of Med. 29, 405-415 (1960).

Honors and Awards relating to this project: None

Serial No. NHI-150
1. Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Biosynthesis of Cholesterol

Principal Investigator: DeWitt S. Goodman

Other Investigators: Dr. George Popjak, Visiting Scientist, National Heart Institute (LCNP).

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.42
Professional: 0.42
Other: 0

Project Description:

Objectives: To investigate the details of the enzymatic synthesis of cholesterol by rat liver, including the definition of the chemical reactions involved, the study of the characteristics of the enzymes catalyzing these reactions, and the study of the mechanisms involved in some of the individual steps.

Methods Employed: In vitro incubation of various combinations of rat liver homogenates with a variety of cofactors and substrates; isolation and separation of the products of the reactions; radioassay of C^{14} and H^3 in various of the products and cofactors.

Patient Material: None

Major Findings: Since returning from London in August, I have been continuing studies, begun there, to define the characteristics of the enzymatic system involved in the cyclization of squalene to sterol. Using C^{14}-labeled squalene biosynthetically prepared in London from 2-C^{14}-mevalonate, it has been found that both the microsome fraction from rat liver homogenates and a soluble protein fraction precipitable with ammonium sulfate between 30 and 60 per cent saturation are required for cyclization. Molecular oxygen and TPNH are necessary; DPNH cannot substitute for TPNH. There is also an almost obligatory requirement for reduced glutathione; this can be partially replaced by the addition

Major Findings (continued)

of ascorbate or by the dialysis of the enzyme fractions against dilute (mM) EDTA. Maximum activity is obtained by the addition of glutathione to a system previously dialyzed against EDTA. The system is strongly inhibited by a number of reagents which are known to affect sulfhydryl groups, including PCMB, arsenite, N-ethyl maleimide, and iodoacetamide. Despite the strength of this inhibition, two closely justaposed -SH groups are apparently not involved, since the inhibition by arsenite is completely reversible by excess glutathione. The important sulfhydryl groups involved in this reaction are associated with the microsomes and not with the soluble protein fraction.

In collaboration with Dr. George Popjak of London, currently a Visiting Scientist with the N.H.I., studies have also been continued to define the mechanism involved in the formation of squalene from farnesyl pyrophosphate. The specific aim of this study has been to determine whether or not protons are lost and/or gained from carbon atoms #1 of farnesyl pyrophosphate during the condensation of two of these sesquiterpenoid intermediates to squalene. The number of protons involved and their source has also been sought. Although this project is still not fully completed, by means of a variety of experiments using deuterium and tritium labeling it has been determined that there is apparently one -and only one- proton lost and then regained at the center of the squalene molecule during its formation. This single proton, which is regained, appears to come from the water of the medium. It thus appears that there is an assymmetrical mechanism involved in the final step of the biosynthesis of squalene.

Significance to Heart Research: This project is part of the basic research program of the National Heart Institute.

Proposed Course of the Project: After completing the problems currently under investigation, it is planned to study in further detail some of the individual reactions involved in the steps between squalene and cholesterol. An attempt will be made to define the normal in vivo course of sterol intermediates involved in this series of reactions.

Part B Included: No.

Serial No. NHI - 151

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Alkaloid Isolation and Structure Studies

Principal Investigator: Helen A. Lloyd, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1
Professional: 1
Other: 0

Patient Days:
None

Project Description:

Progress During the Past Year:

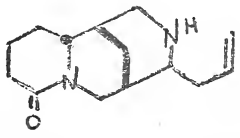
The structures of two alkaloids, jamaidine and jamaicensine, isolated from Ormosia jamaicensis Urb., has been definitely established. Jamaidine $C_{15}H_{24}O_2N_2$ was shown to be 13-epihydroxylupanine I.

I

Although its epimer, 13-hydroxylupanine, has been reported in many plants of the Leguminosae family, this is the first time that epihydroxylupanine has been found in nature.

The structure of jamaicensine $C_{14}H_{22}ON_2$ was determined as 11-allyltetrahydrodesoxycytisine II. Reaction of II with formaldehyde under so-called biogenetic conditions gave jamaidine I in good yield.

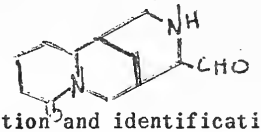
II



CH_2O →

I

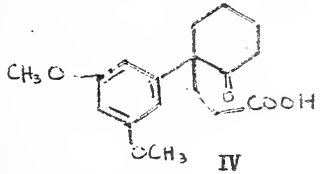
A partial stepwise oxidation of the side chain of II by a novel degradation method was accomplished and yielded the aldehyde III



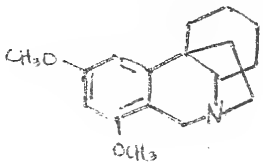
The separation and identification of the Ormosia alkaloids and of a number of Lupin alkaloids by gas chromatography were studied.

Other plant materials were examined. The alkaloidal extract of a species of Brongniartia (Leguminosae) was found to consist mostly of cytisine. In a preliminary study of Astrocasia phyllantoides (Euphorbiaceae), two apparently new alkaloids have been isolated.

Compound IV has been prepared as a step in the synthesis of V, in order



IV



V

to determine the position of a methoxyl group present in a large number of Amaryllidaceae alkaloids.

Direction of Current Research:

The identification and structure determination of alkaloids from Astrocasia phyllantoide and from Brongniartia species will be continued. Other plant materials will be investigated.

The synthesis of compound V is in progress.

Part B included. Yes

Serial No. NHI - 151
1. Laboratory of Chem
of Natural Produc
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Publications

Lloyd, H. A., Fales, H. M., Highet, P. F., VandenHeuvel, W. J. A. and Wildman, W. C., Separation of Alkaloids by Gas Chromatography, J. Am. Chem. Soc., 82, 3791 (1960).

Lloyd, H. A. and Horning, E. C., Alkaloids of Ormosia jamaicensis, J. Org. Chem., 25, 1959 (1960).

Lloyd, H. A., Alkaloids of Ormosia jamaicensis. The Structures of Jamaicensine and Jamaidine, J. Org. Chem. (in press).

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Serial No. NHI - 152
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Characterization of Alkaloids

Principal Investigator: R. J. Highet, Ph.D.

Other Investigators: Helen Marie Walker, B.S.

Cooperating Units: None

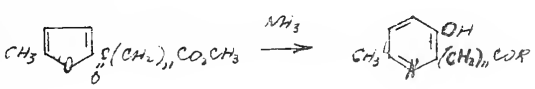
Man Years:
Total: 2.0
Professional: 1.0
Other: 1.0

Patient Days:
None

Project Description:

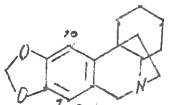
Progress During the Past Year:

(1) Cassine. An extensive study of the products of Hofmann degradations of N-methylcassine has failed to provide a material which can be identified. Therefore, an attempt has been launched to synthesize the structure most likely for dehydrocassine, II. The furan derivative is prepared by a Friedel-Crafts reaction between 2-methylfuran and 12-carbomethoxydodecanoyl chloride. Treatment of I with alcoholic ammonia at 170° has produced material with the spectral properties anticipated of III.



I

II, R = CH_3
III, R = NH_2



IV

In the course of the preparation of cassine a quantity of casselsine suitable for future structural studies has been obtained, as well as small quantities of other alkaloids, as yet uncharacterized.

(2) Alkaloids of the Amaryllidaceae. a. A variety of Amaryllis alkaloids are known to be derivatives of the ring system of powellane, IV

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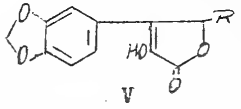
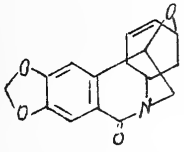
Previous information has not excluded the alternative structure in which the aromatic methoxyl group is located at C-10. A study of the nuclear magnetic resonance spectra of a series of such compounds has now shown structure IVto be correct.

b. It has been discovered that heating 3,4-methylenedioxypyruvic acid produces a compound whose structure is believed to be V, R = piperonyl. Heating equimolar mixtures of the pyruvic acid and aldehydes effects similar condensations. The corresponding compound from 5-hydroxyvaleraldehyde has been prepared (R = $CH_2CH_2CH_2CH_2OH$), promising a intermediate for the synthesis of certain Amaryllis alkaloids.

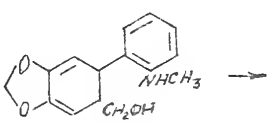


V

(3) Miscellaneous. Structural studies on haemanthidine have been completed by the preparation of nortazettine diacetate and the amino acid produced by hydrolysis of the lactam VI. Pokeweed root has been examined in hopes of finding the alkaloid previously reported; aqueous and alcoholic extraction provides a generous quantity of neutral material, but the alkaloidal content is very small. The structure of ismine VII has been clearly established by the definite identification of the phenanthridone derivative VIII but synthetic studies remain unsuccessful.



VI



VII VIII

Direction of Current Research:

The characterization of alkaloids of the genus Cassia will be continued. Synthetic potentialities of the lactone V will be investigated.

Part B included. No.

Serial No. NHI - 153
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Isolation and Characterization of Components of the Kallikrein System from Human Urine, Pancreas and Blood Plasma.

Principal Investigator: Jack V. Pierce, Ph.D.

Other Investigators: Hiroshi Moriya, Ph.D. (Visiting Scientist)
Patricia Wagner (technical)

Cooperating Units: S. J. Sarnoff, L. C. Sarnoff, M.E. Webster, W. D. Fisher (Laboratory of Cardiovascular Physiology Serial No._____)

Man Years:
Total: 3
Professional: 2
Other: 1

Patient Days:
None

Project Description:

Progress During the Past Year:

Introduction:

With the isolation of pure bradykinin from bovine plasma and its structure determination by Elliott and coworkers in England and its synthesis by Boissonnas and coworkers in Switzerland, an additional impetus has been given that part of our work aiming at the isolation of human plasma kallidin. Bradykinin is a linear nonapeptide containing L-amino acids in the following sequence:

H_2H.Arg·Pro·Pro·Gly·Phe·Ser·Pro·Phe·Arg·COOH

We have obtained two kallidins from the action of human urinary kallikrein on outdated human plasma. They differ in both physical and chemical properties. Kallidin I is contaminated with two inactive peptides to an undetermined extent, but is known to contain arginine, glycine, phenylalanine and serine. Kallidin II may be contaminated by one arginine-containing peptide and contains, besides the amino acids found in I, lysine. The relationship between the kallidins and bradykinin, as well as the nature

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of the Kallidin produced in vivo, have yet to be explored.

Considerable progress has been achieved in the purification of human urinary, pancreatic and plasma kallikreins.

Kallidinogen has received the least emphasis during the year. About 10 liters of human plasma have been taken through the first step and one liter has been purified 50-fold.

Abbreviations

DEAE-C	diethylaminoethyl cellulose
CM-C	carboxymethyl cellulose
HPK	human plasma kallikrein
HUK	human urinary kallikrein
HPanK	human pancreatic kallikrein
ml.*	on the basis of one ml. of starting plasma or urine.

Experimental:

1. Human Plasma Kallidinogen. Previous work showed that the DEAE-C eluate from diluted human plasma could be further purified by chromatography on hydroxyl apatite. However, it was found necessary to subject later preparations to DEAE-C chromatography at pH 6.0 prior to hydroxyl apatite chromatography. The kallidinogen activity, eluted from the DEAE-C column with μ = 0.15 potassium phosphate buffer, pH 6.0, was pooled, dialyzed and freeze-dried. This material was adsorbed to a hydroxyl apatite column and the activity was eluted with μ = .05 to .075 (.026 - .039M) potassium phosphate buffer, pH 6.8. The pooled active fractions were dialyzed, filtered through a fritted glass funnel to remove insoluble material, and freeze-dried. These results are briefly summarized in the following table:

Description of Fraction	Kallidinogen Assay Units/ml.*	Units/mg.
Starting human plasma	1.0	.012
DEAE-C eluate	.50	.06
DEAE-C column eluate	.50	.37
Hydroxyl apatite column eluate	.20	1.65
Dialyzed, filtered and freeze-dried eluate	.08	0.66

The final product contained only about 50 per cent of the activity originally in the hydroxyl apatite eluate. Consequently, in future work alternative methods of desalting this fraction will be tried, such as Sephadex Chromatography.

2. Human Plasma Kallidin. Outdated human plasma, after treatment at pH 3.0 and 37° for 10 min. to destroy kallikrein inhibitors and

kallidinase, was treated with crude human urinary kallikrein at pH 7.4 and 37° for 2 hours. The kallidin thus released was adsorbed to XE-64 at pH 5.0. Elution of inactive materials was accomplished with ammonium formate at pH 6.5, followed by elution of the kallidin with ammonium formate at pH 8.2. The freeze-dried active eluate was extracted with a small amount of water and the extract chromatographed on Sephadex G-25 to separate the kallidin from proteins and other impurities. Chromatography of the active material from the Sephadex column on CM-C at pH 5.0, washing with .01M ammonium formate-.001M EDTA, pH 5.0, and with the same buffer without EDTA and developing with a gradient 0.01M ammonium formate, pH 5.0 to 0.50M ammonium formate pH 7.5, gave two peaks of kallidin activity.

Human Plasma Kallidin: Summary of Fractionation Steps on 20 l. of Outdated Human Plasma

Description of Fraction	Solids	Kallidinogen Units/ml.*	Kallidin Units/ml.*	Kallidin Units/mg.
O.D. human plasma	1,760. g.	0.70		
Acid-treated plasma	"	.46	0.40	
" " " + HUK	1,280. g.		.80	0.012
pH 8.2 eluate of XE-64	1.20 g.		.40	6.7
Sephadex chromatography (1)	84. mg.		.28	66.
" " (2)	26. mg.		.26	200.
CM-C chromatography - Peak I	5.5 mg.		.20	710.
" " - Peak II	3.6 mg.		.06	340.

Hydrolysis of 10 µg. each of kallidins I and II and of "pure" natural bradykinin (provided by Dr. F. Elliott) with 6 N HCl at 105° C for 24 hours and high-voltage paper electrophoresis on the hydrolysates (method of W. J. Dreyer) gave the following results*:

	Amino Acid							
Peptide	Lys	Arg	Gly	Ser	Phe	Glu	Thr	Ala
Bradykinin	-	++++	+++	+++	+	±	±	+
Kallidin I	-	+++++	+++	+++	++	-	-	±
Kallidin II	++	++++	+++	+++	+	+	±	+

It is thus evident that the sample of bradykinin is contaminated to about the same extent as kallidin II but more than I.

* Proline is very probably present in kallidins I and II; however, a suitable technique for detecting proline in Dreyer's method has not yet been worked out.

The results of subjecting the two kallidins to paper electrophoresis in two different systems are shown below:

	System 1			System 2			
	d	N	S	d	N	S	Biol. Activity
Kallidin I	18.4	++++	+++	15.2	++++	+++	+
	19.7	+	-	16.3	+	-	-
	20.7	++	-	17.2	++	+	-
Kallidin II	20.6	++++	+++	16.9	++++	+++	
				18.2	-	+	

System 1: 2M acetic acid; System 2: 1:10:289 pyridine-acetic acid-water

d = cm. from origin N= ninhydrin spray S=Sakaguchi; spray

It is not possible at present to estimate the degree to which the kallidins are contaminated. Experiments are now being done to settle this question, important in that the answer will decide whether or not further purification is necessary before chemical work is undertaken.

It will be observed in the fractionation table that the acid-treated plasma already contained considerable kallidin activity even before treatment with kallikrein. This may account for our having found two kallidins, one produced by acid treatment and the other by treatment with kallikrein. To test this and other possibilities, a purified kallidinogen preparation (DEAE-C eluate described on page 2) will be acid-treated and incubated at pH 7.4 alone and with crude and purified HUK and with HPK; also a sample of human plasma will be acid-treated and then incubated at pH 7.4. The reaction products from treating the kallidinogen preparation with HUK and HPK will be carried through as many of the fractionation steps shown in the table as necessary to provide material suitable for paper electrophoresis whereby kallidins I and II can be distinguished.

3. Human Plasma Kallikrein. Our earlier procedure for preparing this enzyme has been modified and extended. The supernatant from acetone-treated and dialyzed outdated human plasma (3.5 l.) was treated with 140. mg./ml. of DEAE-C (0.7 meq./g.) at pH 6.0. The DEAE-C filtrate was then stirred 1.5 hr. at 15° with 40. mg./ml. of XE-64 at pH 5.0. The resin, recovered by filtration and washed thoroughly with water, was eluted with 0.1M ammonium formate, pH 6.6, then with 0.2M ammonium formate, pH 8.4. The eluates were freeze-dried, extracted with water, and the extracts freeze-dried.

The pH 8.4 eluate, which contained nearly all of the esterase activity recovered, was subjected to ammonium sulfate fractionation at 2°C. The major portion of activity was found between 52 and 70 percent of saturation. This material was desalted and further purified by Sephadex G-75 chromatography. The activity appeared after one void volume, indicating a molecular weight of more than 50,000.

Description of Fraction	TAME Assay Units/ml.*	Units/mg.
Dialyzed and centrifuged acetone-treated plasma	4.32	.064
DEAE-C filtrate	2.34	.148
" eluate	1.08	.019
XE-64 filtrate	0.40	.032
" pH 6.6 eluate	.14	.152
" pH 8.4 "	1.50	2.0
52 to 70% $(NH_4)_2SO_4$ fraction	0.75	
Sephadex G-75 fraction	.56	9.6

4. Human Urinary Kallikrein. Further 150-liter batches of urine have been processed by the procedure described in the two previous annual reports. The crude product, with a potency of 4 to 6 FU/mg., has been fractionated with acetone in the cold. Between 50 and 60 percent (v/v) acetone a material was obtained assaying 16-26 FU/mg. in about 80 percent yield.

Ammonium sulfate fractionation of the acetone product in the range 58 to 72 percent of saturation, followed by Sephadex G-75 chromatography, afforded material with a potency of 120 to 160 FU/mg. in 30 to 50 percent yield.

5. Human Pancreatic Kallikrein. Crude preparations obtained by the method described in our previous annual report have been purified by acetone and ammonium sulfate fractionation. The fraction between 50 and 60 percent (v/v) acetone obtained from material assaying 2.9-5.7 FU/mg. had a purity of about 20 FU/mg. and represented 60 percent of the initial activity.

Fractionation of the acetone product with ammonium sulfate between 60 and 80 percent of saturation gave a product with a potency of about 100 FU/mg. in 90 percent yield.

Direction of Current Research:

Work will be continued on the elucidation of the structures of the kallidins and the comparison of these and the kallidins obtained by the action of human plasma and pancreatic kallikreins on whole plasma and purified kallidinogen, with bradykinin. Probably only one kallidin is formed in vivo (by the action of released plasma kallikrein), hence it will be of great interest to learn its structure.

Attempts will probably be made to obtain pure kallidinogen, which might later prove to be of crucial importance to the kallidin problem.

Further purification of human urinary, pancreatic and plasma kallikreins will be attempted.

Part B included. No.

Serial No. NHI - 154
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Investigations of the Properties of Certain Natural Products.

Principal Investigator: W. C. Wildman, Ph.D.

Other Investigators: Patricia F. Highet (technical)

Cooperating Units: None.

Man Years:
Total: 2.0
Professional: 1.0
Other: 1.0

Patient Days:
None

Project Description:

Selected investigations in the chemical, physical, pharmacological and medicinal properties of various substances, isolated from naturally occurring materials.

Progress During the Past Year:

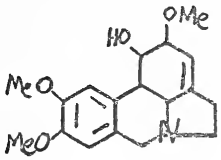
A. Isolation Studies. - Isolation of alkaloids from the King Alfred Daffodil were continued to provide materials for pharmacological and clinical evaluation by Dr. Richard Irwin (NINDB). Attempts to incorporate radioactive tyrosine into Sprekelia formosissima and Nerine bowdenii by root feeding gave no incorporation. Satisfactory results were achieved when leaf feeding was employed with the narcissus "Twink." Lycorine, norpluviine, galanthamine and natalensine have been isolated from these bulbs with significant radioactivity. The metabolic products of the fungi, Pleurotus griseus and P. mutilans have been isolated. From the former, an antibiotic "pleurotin", $C_{20}H_{22}O_5$ has been isolated. P. mutilans affords "pleuromutilin", which may be isolated in radioactive form when the fungus is grown with radioactive mevalonic acid present.

Attempts have been made to determine the composition of various plant extracts by gas phase chromatography. Promising results have been obtained in examinations of the alkaloids of the Amaryllidaceae and Papaveraceae. The GPC behavior of the more common analgesics has been

studied and preliminary studies by GPC of the metabolic fate of various analgesics were completed.

The alkaloids of Haemanthus multiflorus were found to be lycorine and montanine. No haemultine, previously reported in this source was detected.

B. Structural Studies. - Suitable degradative pathways have been sought to determine the site of incorporation of radioactive tyrosine into Amaryllidaceae alkaloids. Hofmann degradation of galanthine (I) yields a mixture of products and it is felt that the Emde degradation offers more



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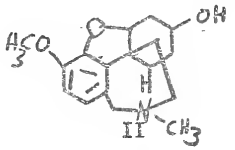

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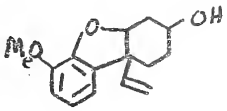
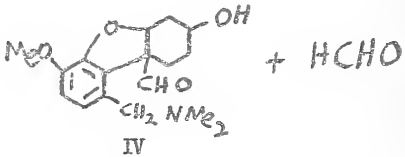


III



IV

promise. Hofmann degradation of galanthamine proceeded in good yield to give a methine. However, the methine could not be degraded selectively in subsequent steps. Hofmann degradation of dihydrogalanthamine (II) (lycoramine) proceeds well to give the methine (III). Hydroxylation of III with osmium tetroxide followed by glycol cleavage gives formaldehyde which was isolated as the dimedone derivative.

Pleurotin, $C_{20}H_{22}O_5$, m.p. 210° dec. has been isolated from Pleurotus griseus. Preliminary degradations, IR, U.V. and N.M.R. Spectra are compatible with its formulation as a quinone containing a γ-lactone group. Pleurotin is unstable to alkali, giving highly colored tars. Reduction of pleurotin with either sulfur dioxide or acidic sodium borohydride gave a crystalline dehydro derivative, m.p. 234-235° dec, which appears to be a hydro quinone.

Direction of Current Research:

The isolation and characterization of alkaloids and other natural products derived from selected plant materials and fungi will be continued. Studies on the biogenesis of these substances will be continued. Applications of gas phase chromatographic and high resolution infrared spectroscopy

techniques to these problems will be emphasized.

Part B included. Yes.

Serial No. NHI - 154
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

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Individual Project Report
Calendar Year 1960

Part B: Publications

Fales, H. M. and Wildman, W. C., The Structures of Haemanthamine and Crinamine, J. Amer. Chem. Soc. 82, 197 (1960).

Highet, P. F. and Wildman, W. C., Hofmann Degradation of 3a-(3,4-Methylenedioxyphenyl)-1-Methyloctahydroindole, J. Org. Chem. 25, 287 (1960).

Lyle, R. E., Kielar, E. A., Crowder, J. R. and Wildman, W. C., Alkaloids of Nerine bowdenii and W. Wats. and Crinum moorei J. D. Hook, J. Amer. Chem. Soc. 82, 2620 (1960).

Warnhoff, E. W. and Wildman, W. C., The Structure and Stereochemistry of Undulatine, J. Amer. Chem. Soc. 82, 1472 (1960).

Fales, H. M. and Wildman, W. C., The Stereochemistry of Amaryllidaceae Alkaloids Derived from 5,10b-Ethanophenanthridine, J. Amer. Chem. Soc. 82, 3368 (1960).

Lloyd, H. A., Fales, H. M., Highet, P. F., VandenHeuvel, W. J. A. and Wildman, W. C., Separation of Alkaloids by Gas Chromatography, J. Amer. Chem. Soc. 82, 3791 (1960).

Wildman, W. C. in The Alkaloids, Vol. VI, R. H. Manske, Ed., Chapter 8, (Colchicine and Related Compounds) and Chapter 9, (Alkaloids of the Amaryllidaceae), Academic Press, Inc., New York 1960.

Battersby, A. R., Binks, J. H. and Wildman, W. C., Biogenesis of Lycorine, Proceedings Chem. Soc. (in press).

Serial No. NHI - 155
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Investigations of Gas Chromatographic Techniques in the Analysis of Complex Substances of Biological Interest.

Principal Investigator: W. J. A. VandenHeuvel, Ph.D.
E. C. Horning, Ph.D.

Other Investigators: Kay V. Anthony (technical)

Cooperating Units: None

Man Years:
Total: 1.35
Professional: 0.85
Other: 0.50

Patient Days:
None

Project Description:

Progress During the Past Year:

The microanalytical separation of steroids and other naturally-occurring compounds of complex structure can now be accomplished by gas chromatographic methods. Current techniques employ relatively thin films of thermostable phases on deactivated supports, with sensitive detectors of the ionization type. These methods were developed on the basis of experience gained in working with fatty acid ester separations. The extension of gas chromatography to steroids was long regarded as an impossibility, largely because of misconceptions based on early experiments. The chromatographic separation of cholesterol from related steroids was accomplished earlier only at high temperatures and with retention times of hours. The retention times currently observed for cholesterol and other sterols is usually about 10-20 minutes, and this may be reduced to a matter of a minute or two if desired.

This very sensitive and powerful method has been applied to a number of classes of compounds of particular interest in biological and biochemical work. These include sterols, sex hormones, bile acids, adrenal cortical steroid hormones and a number of vitamins, drugs and alkaloids.

Additional work has shown that numerous phases of varying degrees of polarity can be used, and that differential analysis involving the use of two phases can be carried out. This has further extended the range of usefulness of the methods.

Direction of Current Research:

Current research is directed toward answering the question of what groups of molecules can be chromatographed; that is, what are the limits of the method in terms of molecular weight and functional groups. An attempt is also being made to investigate the influence of functional groups and stereochemical changes in chromatographic behaviour. Finally, these methods are being applied to the analysis of complex mixtures derived from biological systems.

Part B included. Yes.

Serial No. NHI-155
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Publications

VandenHeuvel, W. J. A., Sweeley, C. C. and Horning, E. C., Separation of Steroids by Gas Chromatography, J. Am. Chem. Soc., 82, 3481 (1960).

VandenHeuvel, W. J. A., Sweeley, C. C. and Horning, E. C., Microanalytical Separations by Gas Chromatography in the Sex Hormone and Bile Acid Series, Biochem. and Biophys. Res. Comm., 3, 33 (1960).

VandenHeuvel, W. J. A. and Horning, E. C., Gas Chromatography of Adrenal Cortical Steroid Hormones, Biochem. Biophys. Res. Comm., 3, 356 (1960).

VandenHeuvel, W. J. A., Sweeley, C. C. and Horning, E. C., "Separation of Steroids by Gas Chromatography," Symposium on Drugs Affecting Lipid Metabolism, Milan, Italy, June 2-4, 1960 (sterols and sterol esters).

VandenHeuvel, W. J. A., Horning, E. C., Sato, Y. and Ikekawa, N., Gas Chromatographic Separation of Steroidal Amines, J. Org. Chem., in press.

Lloyd, H. A., Fales, H. M., Highet, P. F., VandenHeuvel, W. J. A. and Wildman, W. C., Separation of Alkaloids by Gas Chromatography, J. Am. Chem. Soc., 82, 3791 (1960).

Ziffer, H., VandenHeuvel, W. J. A., Haahti, E.O.A. and Horning, E. C., Gas Chromatographic Behavior of Vitamins D_2 and D_3, J. Am. Chem. Soc., in press.

Haahti, E. O. A., VandenHeuvel, W. J. A. and Horning, E. C., Gas Chromatographic Separations of Steroids with Polyester Phases, J. Org. Chem., in press.

Vagelos, P. R., VandenHeuvel, W. J. A. and Horning, M. G., Identification of Hydroxamic Acids by Gas Chromatography of Isocyanate Derivatives, Anal. Biochem., in press.

Serial No. NH1 - 156
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Synthesis, Degradation, Interconversion and Biogenesis of the Amaryllidaceae Alkaloids.

Principal Investigator: Henry M. Fales, Ph.D.

Other Investigators: None

Cooperating Units: None

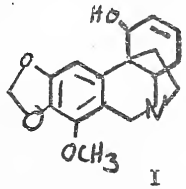
Man Years:
Total: 1
Professional: 1
Other:

Patient Days:
None

Project Description:

Progress During the Past Year:

The complete stereostructure of buphanamine has been shown to be I. The complete stereostructure of hemultin has been shown to be II. A



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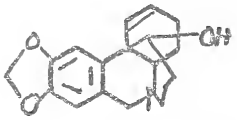


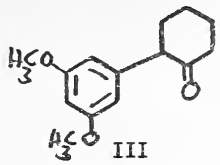
II

report that II has been found to occur naturally has been investigated but could not be confirmed.

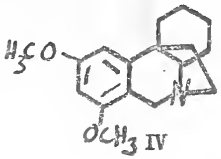
Much effort has been spent on the gas phase chromatography of alkaloids of the Amaryllidaceae and those of the Papaveraceae, especially morphine and codeine. Indole alkaloids serotonin, melatonin and ephedrine-like alkaloids including one catecholamine have been successfully analyzed. Analgesics of the barbiturate group have been analyzed. A technique has been developed to detect compounds being eluted from the gas phase chromatography columns directly by infrared. Several adenosine and a few cytidine

derivatives have been analyzed and in one case an inositol was chromatographed.

Compound III has been synthesized in an effort to prepare IV



III



IV

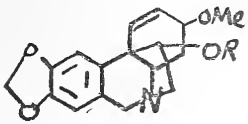
which will prove the location of the methoxyl in some 20 alkaloids of this series.

Norbelladine V has been synthesized and it will now be prepared

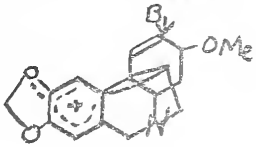
V

in radioactive form for plant feeding purposes. It is expected to be the biological precursor of a large number of alkaloids of the Amaryllidaceae.

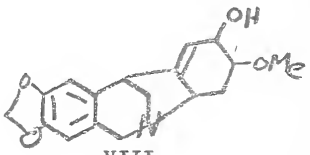
The mechanism of the rearrangement of haemanthamine has been found to involve, in all likelihood VI ⟶ VIII.



VI



VII



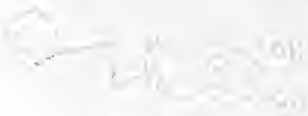
VIII

Direction of Current Research:

The biogenesis of the alkaloids of the Amaryllidaceae is being investigated by incorporating complex C^{14} labelled precursors and degrading the products to locate radioactive carbon. Gas phase chromatography of complex bases is being investigated with a view toward improving resolution and quantitation.

Studies on the infrared spectra of hydrocarbons are being conducted in collaboration with Dr. Joseph Hayes of the Laboratory for Technical Development. By the use of this laboratory's curve analyzer an attempt will be made to analyze hydrocarbons and derivatives (fatty esters) qualitatively in the 3μ region.

Part B included. Yes.



Serial No. NHI - 156
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

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Individual Project Report
Calendar Year 1960

Part B: Publications

Fales, H. M. and Wildman, W. C., The Structures of Haemanthamine and Crinamine, J. Amer. Chem. Soc. 82, 197 (1960).

Fales, H. M. and Wildman, W. C., The Stereochemistry of Amaryllidaceae Alkaloids Derived from 5,10b-Ethanophenanthridine, J. Amer. Chem. Soc. 82, 3368 (1960).

Haahti, E. and Fales, H. M., Continuous Infrared Functional Group Detection of Gas Chromatographic Eluates, Chemistry and Industry (in press).

Lloyd, H. A., Fales, H. M., Highet, P. F., VandenHeuvel, W. J. A. and Wildman, W. C., Separation of Alkaloids by Gas Chromatography, J. Amer. Chem. Soc. 82, 3791 (1960).

Serial No. NHI - 157
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Preparations, Purifications and Special Isolations.

Principal Investigator: David L. Rogerson, Jr.

Other Investigators: James D. Link (technical)
Douglas L. Johnson (technical)

Cooperating Units: Dr. John C. Keresztesy and Henry E. Lutterlough, NIAMD and James M. Miles, NHI. Large scale equipment.

Man Years:
Total: .50
Professional: .20
Other: .30

Patient Days:
None

Project Description:

Progress During the Past Year:

The 100 gallon fermentation unit has been utilized 50 times to grow a variety of microorganisms, particularly Escherichia coli. Due to modifications in the sterilization procedure and an improved mixing assembly, these bacteria have been grown successfully with very few exceptions.

The preparation of Brushite in excess of 10 kilograms and the isolation of transesterified triglycerides have been completed. Other preparations include the syntheses of two polymers, the washing and grading of absorbents in large quantities, particularly silicic acid, and the purification of large volumes of solvents for chromatographic uses.

Approximately 50 gallons of normal urine have been collected from NIH employees and desired constituents have been isolated. Also, relatively large volumes of urine collected from patients have been concentrated, dialyzed and lyophilized for investigators in other laboratories and Institutes.

Direction of Current Researches:

Due to increased interest in heat labile compounds and solutions, processing procedures and facilities are being evaluated for possible alteration or replacement.

Part B included. No.

Serial No. NHI - 158
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Testing and/or Isolation of Alkaloids, Glycosides and Other Constituents from Plant Materials.

Principal Investigator: David L. Rogerson, Jr.

Other Investigators: James D. Link (technical)
Douglas L. Johnson (technical)

Cooperating Units: Dr. B. G. Schubert, Plant Industry Station, U.S. Department of Agriculture, Beltsville, Md. Plant Procurements and Identifications. Dr. J. C. Keresztesy and Henry E. Lutterlough, NIAMD and James M. Miles, NHI. Large Scale Equipment.

Man Years:
Total: .50
Professional: .20
Other: .30

Patient Days:
None

Project Description:

Progress During the Past Year:

Twenty-three plant samples weighing a total of 1500 pounds have been received during the past year. Nine of the samples received were new observations and seven of these gave positive alkaloid tests. In addition, samples from each of twenty-four previously received plants were processed and tested for glycosides and moderate to very noticeable activity or death in mice was observed in eight of the tests.

Plant materials which have been processed for alkaloids include twelve members of the Amaryllidaceae weighing a total of 275 pounds and twenty-six miscellaneous plant samples totaling 95 pounds. Also, 1000 pounds of Rhododendron maximum are being processed andromedotoxin. Twenty opium samples supplied by United Nations were processed for special study.

Direction of Current Researches:

Available plant materials which contain alkaloids will be processed on a one pound scale and the resulting extracts will be investigated employing the gas chromatograph. Also, glycoside testing will continue using available plant materials which do not contain alkaloids.

Part B included. No.

Serial No. NHI - 159
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: An Investigation of Stationary Phases for Gas-Liquid Chromatography.

Principal Investigator: C. C. Sweeley, Ph.D.
E. C. Horning, Ph.D.

Other Investigators: Kay V. Anthony (technical)
Kay Y. Maddock (technical)

Cooperating Units: None

Man Years:
Total: 1.5
Professional: 0.5
Other: 1.0

Patient Days:
None

Project Description:

Progress During the Past Year:

Work during the year (until June) was devoted to two aspects of lipid chemistry. Methods for the separation of phospholipid classes were investigated with a view to applying these procedures to blood and other tissue. Silicic acid was found to be the most satisfactory column material, and suitable procedures were found for blood phospholipids separations. Gas chromatographic work was continued with a view to developing methods suitable for the chromatography of steroids. Two thermostable stationary phases were investigated. These were ethylene glycol isophthalate and the silicone polymer SE-30 of the General Electric Co. Both gave excellent results with relatively large molecules at temperatures in the range 230-270°. The use of lower temperatures followed from subsequent experiments in which relatively thin films were used.

The first practical separations of steroids at moderate temperatures (200-220°) was demonstrated with an SE-30 phase in work with Dr. W. J. A. VandenHeuvel.

Direction of Current Work:

Current work is being carried out by Dr. W. J. A. VandenHeuvel and Dr. E. O. A. Haahti.

Part B included. Yes.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Publications

VandenHeuvel, W. J. A., Sweeley, C. C. and Horning, E. C., Separation of Steroids by Gas Chromatography, J. Am. Chem. Soc., 82, 3481 (1960).

VandenHeuvel, W. J. A., Sweeley, C. C. and Horning, E. C., Microanalytical Separations by Gas Chromatography in the Sex Hormone and Bile Acid Series, Biochem. Biophys. Res. Comm., 3, 33 (1960).

Sweeley, C. C. and Horning, E. C., Microanalytical Separation of Steroids by Gas Chromatography, Nature, 187, 144 (1960).

VandenHeuvel, W. J. A., Sweeley, C. C. and Horning, E. C., Separation of Steroids by Gas Chromatography "Symposium on Drugs Affecting Lipid Metabolism" (Milan, Italy, June 2-4, 1960), Ed., S. Garattini, in press.

Serial No. NHI -160
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Investigations of Human Skin (Sebum) Lipids

Principal Investigator: E. O. A. Haahti

Other Investigators: K. C. Maddock

Cooperating Units: None

Man Years:
Total: 1.3
Professional: 0.8
Other: 0.5

Patient Days:
None

Project Description:

Progress During the Past Year:

Methods for the study of skin (sebum) lipids have been developed and applied to human materials. The initial separation is carried out by silicic acid chromatography. Neutral lipids are fractionated into the following classes: (a) saturated hydrocarbons, (b) unsaturated hydrocarbons, (c) wax esters (including cholesterol esters), (d) triglycerides, (e) sterols, and (f) fatty acids. Additional methods for separating long-chain wax esters from cholesterol esters are under investigation.

Previous investigations of sebum have failed to yield definitive answers on composition because of inadequacies of technique. The chief difficulty was the past inability to carry out direct gas chromatographic separations of high molecular weight compounds. This has been resolved by the development of new methods, including the use of temperature programming with thermostable polyester phases. With neopentyl glycol succinate, for example, it has been possible to carry out a direct separation of human long-chain wax esters. The major component is a C_{36} ester. The techniques are useful for studying high molecular weight acids, alcohols and esters. Sterols may be separated either as free alcohols or as acetyl derivatives.

This work required the development of a new design of gas chromatography apparatus. A coiled glass column is used in a small container arranged for temperature programming. An apparatus for following

The elution of components by infrared analysis has also been developed in collaboration with Dr. H. M. Fales.

Direction of Current Research:

Additional studies of methods for the separation of sterols, wax esters and other high molecular weight compounds are projected. Studies will be made particularly of additional ways of using polar phases. Analytical investigations of sebum composition will be continued.

Part B included. Yes

PHS-NIH
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Part B: Publications

Haahti, E. O. A., VandenHeuvel, W. J. A. and Horning, E. C., Gas Chromatographic Separations of Steroids with Polyester Phases, J. Org. Chem. (in press).

Ziffer, H., VandenHeuvel, W. J. A., Haahti, E. O. A. and Horning, E. C., Gas Chromatographic Behavior of Vitamins D_2 and D_3, J. Am. Chem. Soc. (in press).

Haahti, E. O. A. and Fales, H. M., Continuous Infrared Functional Group Detection of Gas Chromatographic Eluates, Chemistry and Industry (in press).

Serial No. NH1 - 161
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Basic Chemistry of Biological Reactions

Principal Investigator: E. C. Horning, Ph.D.

Other Investigators: None

Cooperating Units: J.C. Craig, Ph.D., University of Sydney, Sydney, Australia

Man Years:
Total: 0.1
Professional: 0.1
Other: 0

Project Description:

Progress During the Past Year:

A. Amine oxide reactions. Further investigations of the iron-catalyzed rearrangement reaction of amine oxides led to evidence for the transient formation of Fe^{IV} in the intermediate complex. A series of metal-organic complexes were studied as catalysts for the rearrangement through collaboration with Dr. J. C. Craig and the Australian inorganic chemist Dr. F. Dwyer.

B. Hemiacetal oxidation. This reaction was studied as a model for the metabolic formation of certain short-chain and long-chain esters. The basic chemistry was investigated. The reaction is under further study to determine the limitations of structure for the reacting units.

Direction of Current Research:

The work has been suspended pending completions of Dr. Craig's move to the University of California. It will be resumed later.

Part B included. Yes.

Serial No. NHI - 161
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

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Calendar Year 1960

Part B: Publications

Craig, J. C., Dwyer, F. P. Glazer, A. N and Horning, E. C., Tertiary amine Oxide Rearrangements. Part I. Mechanisms. J. Am. Chem. Soc., in press.

Craig, J. C. and Horning, E. C., Preparation of Esters by Hemiacetal Oxidation, J. Org. Chem., in press.

Serial No. NHI-162
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Sex Difference in Histamine Metabolism in Rats

Principal Investigators: Dr. P.A. Shore
Dr. K.J. Netter (Visiting Scientist, University of Hamburg, Hamburg, Germany)

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: During the past few years it has been found that there exist at least two pathways of histamine metabolism. One of these is oxidative deamination of the histamine molecule by the action of diamine oxidase (in mammals identical with "histaminase"). The other, more recently discovered route is N methylation of the imidazol ring of histamine by an enzyme, imidazol-N-methyl transferase. The relative importance of these pathways appears to be dependent upon species, sex, and histamine levels.

A marked sex difference in rats has been described. Male rats have been reported to metabolize histamine chiefly by N methylation while female rats appear to utilize chiefly diamine oxidase. We are attempting to discover the enzymatic basis for this sex difference.

Major Findings: The sex difference in histamine metabolism does not appear to be due to a difference in the activity of the major histamine metabolizing enzymes (diamine oxidase; Imidazol-N-methyl transferase) as measured in vitro. The sex difference

involves histamine catabolism rather than biosynthesis, since female rats excrete unchanged a large part of an injected dose of histamine while male rats excrete only a small fraction of the injected dose. Measurement of the rate of histamine disappearance from blood after its intravenous administration has revealed that removal of the methylating enzyme (by nephrectomy, since the kidney contains almost all of the enzyme in the rat) has no effect on the rate of disappearance in either sex. Blockade of diamine oxidase by administration of aminoguanidine has a definite but equal effect in the two sexes, indicating that in this species, diamine oxidase is of the greater importance in histamine metabolism. After a combination of nephrectomy and aminoguanidine treatment, males exhibit a reduced but still significant metabolism of histamine, but females are almost incapable of destroying histamine. The results suggest that males may have either a third enzyme for histamine metabolism or a source of methylating enzyme which females lack.

Significance to bio-medical research and the program of the Institute: This project is designed to help elucidate physiological factors important in the metabolism of histamine, a substance with marked effects on the cardiovascular system.

Proposed Course of Project: We plan to investigate the possibility that males may have a greater acetylating enzyme activity than do females.

Part B included: No

Serial No. NHI-163
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Histamine Binding and Release

Principal Investigators: Dr. P.A. Shore
Dr. M.E. Tidball (NIH Postdoctoral Fellow)

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.3
Professional: 1.3
Other: 0

Project Description:

Objectives: We are continuing studies on the release of histamine, with current emphasis on release from platelets. Last year we reported finding that both reserpine and serotonin release histamine from rabbit platelets in vivo, but not in vitro.

These studies have led us into a study of factors present in plasma or serum which act to release histamine from platelets. As a corollary, we hope to learn more of the mechanisms by which drugs and other agents affect the binding of amines within other cells as well.

Major Findings: Addition of dilute defibrinated serum freshly prepared from platelet-free plasma, to rabbit platelets suspended in saline causes release of histamine from the platelets. Release is prevented by an excess of ethylenediaminetetraacetate (EDTA) or by a low concentration of heparin. Little release is observed when aged serum is substituted for fresh. Addition of small amounts of thrombin to platelets in dilute aged serum or in 4% albumin solution buffered to pH 7.4 causes release. Thrombin-induced release in serum or in albumin can be blocked by heparin. The results demonstrate that thrombin is a potent releaser of platelet histamine and suggest that thrombin generation in fresh serum is responsible for

release of histamine from platelets.

This demonstration of histamine release in platelets is analogous to the release of histamine from mast cells during anaphylaxis and suggest that release of histamine from various types of cells might proceed by similar mechanisms.

Significance to bio-medical research and the program of the Institute: This project is designed to elucidate the factors involved in the binding and release of histamine from certain tissues.

Proposed Course of Project: We plan to investigate further the calcium dependent mechanism in histamine release and to explore the possibility that thrombin is actively involved in platelet changes in anaphylaxis.

Part B included: Yes

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Individual Project Report
Calendar Year 1960

Part B

Honors and Awards: None

Publications:

Burkhalter, A., Cohn, V.H. and Shore, P.A., Effect of Reserpine, Serotonin and 5-Hydroxytryptophan on Rabbit Platelet Histamine In Vivo and In Vitro. Biochem. Pharmacol. 3, 328 (1960).

Serial No. NHI-164
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Physiologic Disposition and Metabolism of Methaminodiazepoxide (Librium)

Principal Investigator: Dr. P.A. Shore

Other Investigator: Miss R. Gastel Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.1
Professional: 0.05
Other: 0.05

Project Description:

Objectives: Methaminodiazepoxide has been introduced recently into clinical practice as an agent to relieve anxiety states, and is already used widely as a tranquilizing agent. Because of the continued interest of this laboratory in studies on the physiological disposition of drugs, we have initiated studies into the disposition and metabolism of this drug.

Major Findings: A sensitive colorimetric method for the assay of this drug in tissues has been developed, based on cleavage of the drug to form 2-amino-5-chlorobenzophenone which is then diazotized and coupled with Bratton-Marshall reagent to form a red chromophore.

Significance to bio-medical research and the program of the Institute: These studies should provide us with valuable information concerning the chemical pharmacology of a new and important drug.

Proposed Course of Project: We plan to study the rate and routes of metabolism of methaminodiazepoxide and to identify metabolic products if possible.

Part B included: No

Serial No. NHI-165
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Diamine Oxidase and Diamine Oxidase Inhibitors

Principal Investigators: Dr. P.A. Shore
Mr. V.H. Cohn

Other Investigators: None Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.3
Professional: 0.3
Other: 1.0

Project Description:

Objectives: Although the existence of the enzyme diamine oxidase has been long recognized, the nature of its function remains essentially unknown. This project has been carried out in an effort to learn more of the nature of this enzyme and it's substrates and of substances which influence its activity.

Major Findings: Sensitive techniques have been developed for the assay of low levels of diamine oxidase. This has been made possible by development of microfluorometric techniques for the estimation of histamine and agmatine, both of which can now be easily assayed in sub-microgram amounts. Investigation of the kinetics of metabolism of histamine or agmatine at low concentrations has revealed that the affinity of these amines for the enzyme is much greater than has been reported previously by other investigators. Thus we have found a Km of the order of 10^{-6} M instead of values in the order of 10^{-4} M - 10^{-5} M reported elsewhere.

Comparison of the rates of metabolism of the two substrates indicates that the enzyme which metabolizes histamine and agmatine is one and the same. This has been demonstrated by measurement of the ratio of histamine to agmatine metabolism rates in enzyme preparations from different mammalian sources and by studies on the comparative inhibition by various enzyme inhibitors such as amino-

guanidine and murexine. The inhibitor studies have also shown that most of the currently used monoamine oxidase inhibitors also block diamine oxidase. This activity may be associated with the hydrazine moiety since at least one potent monoamine oxidase inhibitor (phenylcyclopropylamine) has no effect on diamine oxidase. This observation has been used to show that contrary to reports of other investigators, monoamine oxidase is incapable of metabolizing histamine.

Significance to bio-medical research and the program of the Institute: These studies are relevant to investigations into the metabolism and function of histamine and other amines which can affect the cardiovascular system.

Proposed Course of Project: We plan to continue the same lines of investigation and also to study the reported effects of alteration of endocrine function on diamine oxidase activity.

Part B included: Yes

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Calendar Year 1960

Part B

Honors and Awards: None

Publications:

Shore, P.A., and Cohn, V.H., Comparative Effects of Monoamine Oxidase Inhibitors on Monoamine Oxidase and Diamine Oxidase. Biochem. Pharmacol., in press.

Shore, P.A., Biochemistry of Monoamine Oxidase Inhibitors, Am. J. Cardiol., in press.

Burns, J.J., and Shore, P.A., Biochemical Effects of Drugs, Ann. Rev. Pharmacol., in press.

Serial No. NHI-166
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Development of Chemical Assay Methods for the Determination of Natural Body Constituents

Principal Investigator: Dr. Roger P. Maickel

Other Investigator: None

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: 0.2
Professional: 0.2
Other: 0

Project Description:

Objectives: The investigation of biochemical behavior requires the use of assay methods for various natural body constituents, including protein hormones, steroids, carbohydrates, fats, amino acids, and various enzymes. In many instances, published methods for these substances are unsatisfactory for use in this research program, because of low sensitivity, lengthy procedures, or lack of specificity. Some methods are amenable to modification to suit the needs of the project, but completely new methods must be developed for some substances. By applying techniques we have used in development of assay methods for drugs, many methods can be devised for the assay of natural body constituents.

Methods Employed:
1) A rapid procedure was developed for the determination of ascorbic acid in tissues. This new method is particularly valuable in the determination of adrenal ascorbic acid.
2) Existing methods for plasma free fatty acids and liver tryptophane peroxidase were modified to increase sensitivity and reproducibility.
3) A published method for the determination of corticosterone in plasma was adapted for use in measuring the output of steroids from adrenal cortical tissue in vitro.
4) A method for the determination of heart muscle phosphorylase has been successfully adapted for determining liver phosphorylase.

Significance to the Program of the Institute: Specific, sensitive assay methods are of obvious importance in the development of a successful program of biomedical research.

Proposed Course of Project: As the need arises, new methods will be developed or existing methods will be modified to permit rapid determination of various natural body constituents in microgram quantities.

Part B included: Yes

Serial No. NHI-166
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Maickel, Roger P.: A Rapid Procedure for the Determination of Adrenal Ascorbic Acid. "Anal. Biochem.", in press.

Honors and Awards relating to this project:

Maickel, Roger P. and Brodie, Bernard B.: A Rational, Organized Approach to the Development of Methods for the Determination of Drugs in Body Fluids and Tissues. Presented at Eastern Analytical Symposium, New York City, November 2-4, 1960.

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Serial No. NHI-167
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in Biochemical Behavior
I. Comparative Effects of Drugs and Environmental Change on ACTH Output.

Principal Investigator: Dr. Roger P. Maickel

Other Investigators: Dr. Milton T. Bush
Dr. W. Robert Jondorf
Dr. Rene R. Martel
Dr. Robert L. Smith
Dr. Erik O. Westermann

Cooperating Unit: Dr. Bush is a Public Health Service Research Fellow.

Man Years (Calendar Year 1960):
Total: 0.2
Professional: 0.2
Other: 0

Project Description:

Objectives: Behavior in unicellular organisms is manifested by adaptation of biochemical reactions to the stimuli of environmental changes. In the mammal biochemical reactions also adapt to environmental change, but the information about the outside world must be relayed from sensory organs to the brain. Accordingly, the brain must coordinate the mechanisms which change the activity of biochemical reactions in response to sensory input. In this manner the brain controls not only psychological and physiological behavior but biochemical behavior as well. One way in which the brain controls the body biochemistry is through the hormonal output of the pituitary gland - the pituitary hormones influence enzyme activities directly or through hormones released from the other endocrine glands. In addition the brain influences body biochemistry through the autonomic nervous system.

The present project has a number of objectives:

1) The nature of and reason for biochemical changes occurring in response to changes in environment.

2) Comparison of biochemical responses to environmental change and to drugs which interact with the hypothalamus-pituitary-adrenal system.

3) The nature of "irreversible" changes, which, occurring with persistent environmental changes or with drugs, might result in chronic diseases.

4) The role of the hypothalamus and other parts of the brain in control of pituitary output.

5) A study of drugs which inhibit ACTH hypersecretion induced by drugs or environmental change.

Methods Employed: Adrenal ascorbic acid was determined by measuring the reduction of ferric chloride in the presence of α,α'-dipyridyl (Maickel, R.P., Anal. Biochem., in press); plasma corticosterone was assayed spectrophotofluorometrically (Guillemin, R., et al., J. Lab. Clin. Med. 54, 830, 1959); plasma FFA levels were determined by a modification of the two phase acid-base titration of Dole (Dole, V.P., J. Clin. Invest. 35, 150, 1956); and liver TPO was measured according to the procedure of Knox (Knox, W.E., Brit. J. Exper. Path. 22, 462, 1951).

Major Findings: A number of ways of measuring hypothalamo-hypophyseal-adrenocortical responses have been investigated.

1) The commonly used index, the fall in adrenal ascorbic acid indicates intensity of pituitary-adrenal stimulation but not its duration, since the ascorbic acid level falls rapidly and there is a lag of several hours before the level returns to normal.

2) Since the rise in plasma corticosterone is not only rapid but persists at levels $>$ 200% above normal as long as ACTH hypersecretion is maintained, this is a delicate index of both intensity and duration of pituitary-adrenal stimulation.

3) Plasma levels of free fatty acids (FFA) generally rise rapidly and remain elevated (100 to 200% above normal) while the stimulus lasts.

4) The rise in liver TPO is a measure of the cumulative effects of pituitary-adrenal stimulation. Maximal activity (200-300% above normal) is reached in 8 to 10 hours and after the stimulus ceases at least 6 hours elapses before the activity returns to normal. Despite the lag in onset of the TPO elevation it seems to be the most sensitive index of a sustained stimulus.

The results obtained from exposure of animals to stressful stimuli such as a low ambient temperature or the pain of an intradermal injection of formaldehyde were similar to those seen in animals given drugs such as reserpine, chlorpromazine, ethanol, amphetamine, and hexobarbital.

Significance to the Program of the Institute: Endocrine mechanisms which control biochemical reactions of the body are an important part of biochemical behavior. An understanding of these mechanisms may be of value in the study of chronic diseases such as atherosclerosis, liver disease etc.

Proposed Course of Project:

1) Other responses of the pituitary to various drugs will be studied. These results will be compared with those produced by physiological stimuli.

2) Biochemical behavior will be described in terms of peripheral biochemical mechanisms which respond to stimuli.

Part B included: No

Serial No. NHI-168
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in Biochemical Behavior
II. The Action of Reserpine and Other Rauwolfia Alkaloids on the Pituitary-Adrenocortical System of the Rat.

Principal Investigators: Dr. Erik O. Westermann
Dr. Roger P. Maickel

Other Investigator: Mr. Samuel A. Alexander

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: 1.0
Professional: 0.7
Other: 0.3

Project Description:

Objectives: Reserpine and other pharmacologically active Rauwolfia alkaloids produce pharmacological effects through the release of biogenic amines in the central nervous system. Previous studies have shown that reserpine elicits a pituitary-adrenal response characterized by a decreased level of adrenal ascorbic acid, increased plasma levels of corticosterone and free fatty acids (FFA), and an elevated activity of liver tryptophan peroxidase (TPO). Further studies were designed to explore the interrelationship of reserpine actions on brain amine levels and the secretion of ACTH by the pituitary.

Methods Employed: Standard assay methods and pharmacological procedures were used.

Major Findings: 1) Reserpine elicits pituitary-adrenal stimulation in any combination of dosage that depletes brain amines by 50%, when sedation is also produced. Among a number of Rauwolfia alkaloids, only those compounds which release brain amines and produce sedation also elicit the pituitary-adrenal response. Isoreserpine which is pharmacologically inactive and does not affect brain amines, does not produce pituitary-adrenal stimulation at the LD_{50} dose (50 mg/kg). Isoraunescine is also inactive.

2) Large doses of reserpine (> 2.5 mg/kg, i.v.) lower pituitary ACTH, in 20 hours by ~70%. The animals with a "depleted" pituitary are incapable of response to a cold environment and in fact a number of the animals die. Thus the reported action of reserpine in "blocking" a stress response is due to exhaustion of ACTH.

3) Meta-hydroxy-3-methyltyrosine, a compound which selectively releases norepinephrine but not serotonin, neither produces sedation nor releases ACTH. Thus the effect of reserpine on the pituitary is due either to the pharmacological action per se or to the change in brain serotonin.

Significance to the Program of the Institute: Since reserpine and reserpine-like compounds find widespread clinical use in the treatment of hypertension and mental disorders, it is of interest to examine the effects of these drugs on endocrine systems.

Proposed Course of Project: 1) A further examination of the relationship between the pharmacological actions of reserpine and its action on the pituitary-adrenal system is necessary. The increased secretion of ACTH in response to administration of reserpine may be due to the release of brain amines or to the sedation produced by the drug.

2) The interrelationships of reserpine and other pharmacological agents which are reputed to "block" a stress response will be examined.

3) Reserpine will be employed as a "tool" to study various endocrine functions.

Part B included: Yes

Serial No. NHI-168
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
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Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Brodie, Bernard B., Maickel, Roger P., and Westermann, Erik O.: Action of Reserpine on Pituitary-Adrenocortical System Through Possible Action on Hypothalamus. Proc. 4th Int. Neurochem. Sym., Varenna, Italy, June 13-17, 1960, in press.

Brodie, Bernard B., Westermann, Erik O., and Maickel, Roger P.: Action of Reserpine on the Hypothalamo-Pituitary-Adrenocortical System. "Proc. Sym." Tech. for Study of Pschyotropic Drugs, Department of Pharmacol, University of Bologna, Bologna, Italy, June 26-27, 1960, in press.

Serial No. NHI-169
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in Biochemical Behavior
III. Responses of the Pituitary-Adrenal Axis of Rats to the Administration of 20-Methylcholanthrene.

Principal Investigators: Dr. W. Robert Jondorf
Dr. Roger P. Maickel

Other Investigator: None

Cooperating Units: None

Man Years (Calendar Year 1960):
Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: Preliminary studies indicated a prolonged response of the pituitary-adrenal axis to administration of the carcinogenic compound, 20-methylcholanthrene. In view of other work from this laboratory demonstrating that 20-methylcholanthrene induces drug enzymes in liver microsomes, further studies of this and similar compounds were considered necessary.

Methods Employed: Standard assay procedures and pharmacological methods were employed.

Major Findings: Doses of as little as 1 γ of 20-methylcholanthrene per rat evoked a measurable pituitary-adrenal response. After administration of 10 mg of the drug per rat, measurable pituitary-adrenal stimulation was observed for 24-36 hours. Somewhat anomalous behavior was observed, since liver tryptophan peroxidase activity was only slightly elevated ($< 120\%$) despite continuously elevated (40-60%) levels of plasma corticosterone. The pituitary-adrenal stimulation produced by 20-methylcholanthrene did not deplete the pituitary of ACTH, since the application of a second stressful stimulus 24 hours after administration of the carcinogen produced a typical pattern of pituitary-adrenal stimulation.

Significance to the Program of the Institute: The action of carcinogenic compounds such as 20-methylcholanthrene on the endocrine glands might have some role in the production of chemical carcinogenesis.

Proposed Course of Project: Further studies of the action of 20-methylcholanthrene on endocrine systems are proposed. Attempts will be made to correlate hormonal activity with specific changes in biochemical mechanisms. The relatively poor pituitary-adrenal response to this compound might be related to induction of liver microsomal enzymes that metabolized corticosterone.

Part B included: No

Serial No. NHI-170
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in Biochemical Behavior
IV. Effects of Chlorpromazine and Other Phenothiazine Tranquilizing Agents on the Pituitary-Adrenal System.

Principal Investigators: Dr. Robert L. Smith
Dr. Roger P. Maickel

Other Investigator: None

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: 0.6
Professional: 0.6
Other: 0

Project Description:

Objectives: Chlorpromazine and other phenothiazine derivatives are widely used as tranquilizing agents. Several reports have suggested that phenothiazine tranquilizers are capable of blocking the response of animals to various non-specific stresses. In view of the known ability of these compounds to decrease central nervous system activity, it is of interest to examine their action on the hypothalamo-pituitary-adrenocortical axis.

Methods Employed: Standard assay procedures and pharmacological methods were used.

Major Findings: Administration of chlorpromazine (5-10 mg/kg, i.p.) to rats produced the typical syndrome of pituitary-adrenal stimulation: decreased adrenal content of ascorbic acid, increased plasma levels of corticosterone and free fatty acids, and increased activity of liver tryptophan peroxidase. A study of dose-response relationships showed that only doses of chlorpromazine which produced sedation ($>$ 2.5 mg/kg) evoked the hypersecretion of ACTH. This dose level also corresponds to the quantity of chlorpromazine necessary to potentiate barbiturate hypnosis. Chlorpromazine sulfoxide, which does not elicit sedation or potentiate barbiturate hypnosis in rodents, did not produce ACTH hypersecretion unless given in toxic doses.

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Significance to the Program of the Institute: In view of the widespread clinical use of chlorpromazine and other phenothiazine tranquilizers, a study of the action of these drugs on the pituitary-adrenal system and other endocrine systems is of obvious value.

Proposed Course of Project:

1) Other phenothiazines, both with and without tranquilizing action, will be investigated for their effects on the pituitary-adrenal system.

2) Further studies will be undertaken in an attempt to elucidate the mechanism of pituitary stimulation by chlorpromazine.

3) Studies will be made of the reported ability of chlorpromazine and other tranquilizers, to block non-specific "stress".

Part B included: No

Serial No. NHI-171
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in Biochemical Behavior
V. Central Nervous System Stimulants and Their Effect on Secretion of ACTH by the Pituitary.

Principal Investigators: Dr. Milton T. Bush
Dr. Roger P. Maickel

Other Investigator: None

Cooperating Unit: Dr. Bush is a Public Health Service Research Fellow.

Man Years (Calendar Year 1960):
Total: 0.4
Professional: 0.4
Other: 0

Project Description:

Objectives: Clinical observations in patients undergoing electroshock therapy have shown increased urinary excretion of 17-ketosteroids. Changes observed in the function or size of various endocrine glands suggested that the central nervous system stimulation produced by electroshock treatment was capable of affecting hormonal output from the pituitary. An investigation of the actions of other central nervous system stimulants on the pituitary was undertaken with the objective of gleaning further knowledge about endocrine function in response to drugs. Preliminary studies suggested the pituitary-adrenal system to be a suitable starting point.

Methods Employed: Standard assay methods and pharmacological procedures were used.

Major Findings: Following treatment of rats with electroshock, the typical responses of pituitary-adrenal stimulation were observed: elevated levels of plasma corticosterone, and decreased adrenal ascorbic acid content. However, plasma levels of free fatty acids were lowered, presumably due to the elevation in blood glucose produced by the stimulation. Central nervous system stimulants such as picrotoxin and metrazol, are also capable of evoking a hypersecretion of ACTH, even when given in sub-convulsive doses. Amphetamine produces pituitary-adrenal stimulation, but only at dose levels somewhat above those necessary to show pharmacological activity.

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Significance to Program of the Institute: The effects of central nervous system stimulants upon pituitary-adrenal function is a further step in the study of biochemical behavior.

Proposed Course of Projects:

1) Further studies will be made of pituitary-adrenal activation by other central nervous system stimulants such as lysergic acid diethylamide, strychnine, and megamide.

2) Experiments will be designed to study the effect of pharmacological antagonists to these stimulants on the pituitary-adrenal axis.

Part B included: No

Serial No. NHI-172
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in Biochemical Behavior
VI. Inhibition of Brain Monoamine Oxidase and Pituitary Function.

Principal Investigators: Dr. Rene R. Martel
Dr. Roger P. Maickel

Other Investigator: None

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: 0.3
Professional: 0.3
Other: 0

Project Description:

Objectives: Compounds which inhibit monoamine oxidase (MAO) in brain are of obvious importance in studies of centrally-occurring events. The brains of animals treated with MAO inhibitors contain abnormally high levels of serotonin and norepinephrine. The high levels of these amines not only effect the behavior of the animals, but also have a marked action on their responses to other drugs. In view of these facts, the finding that reserpine which lowers brain amines causes hypersecretion of ACTH, and of reports that iproniazid (a known inhibitor of MAO activity) affects the level of circulating 17-ketosteroids in the monkey, a study of the relationship between MAO inhibition and pituitary function was instituted.

Methods Employed: Standard assay methods and pharmacological procedures were used.

Major Findings: Preliminary findings show that iproniazid (Marsilid) at a dose of 80 mg/kg, i.p., produces a short-lasting hypersecretion of ACTH from the pituitary presumably owing to a direct effect of the drug. However, MO 911 at a dose of 20 mg/kg, i.p. inhibits brain MAO by $\geq$ 80% (as measured in vitro) within 1 hour, and evokes only a transient, acute hypersecretion of ACTH from the pituitary.

The pituitary-adrenal response of rats to cold stress or to chlorpromazine is not affected by pretreatment with iproniazid 16-18 hours previously.

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The responses to a large dose of reserpine are decreased by 50-60% in animals pretreated with iproniazid. In similar experiments, animals pretreated with MO 911 for 48 hours showed a normal response to cold exposure, although the response to reserpine was decreased by about 33%.

Significance to the Program of the Institute: Monoamine oxidase is an important enzyme in the biochemical regulation of brain function. Changing levels of brain amines may be of importance in understanding the control of hormonal secretion by the pituitary.

Proposed Course of Project: Further studies of the effect of MAO inhibition on pituitary-adrenal output will be made. In particular the relationship of brain amine metabolism to hypothalamic control of the pituitary hormones will be examined.

Part B included: No

Serial No. NHI-173
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in Biochemical Behavior
VII. Derangement of Lipid Mobilization and Transport by Ethanol - The Role of the Pituitary-adrenal System.

Principal Investigator: Dr. Roger P. Maickel

Other Investigators: This work is being carried on in collaboration with Dr. Harriet M. Maling (NHI-LCP) and Dr. Marjorie G. Horning (NHI-LCPM).

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: 0.2
Professional: 0.2
Other: 0

Project Description:

Objectives: Administration of large oral doses of ethanol to rats results in a marked deposition of neutral fat in the liver within 8-12 hours. This increase in liver triglycerides does not occur in hypophysectomized animals suggesting a possible role of the pituitary. Preliminary results suggested that ethanol causes hypersecretion of ACTH in rats. A more detailed study of this response and of its relationship to the triglyceride mobilization was undertaken.

Methods Employed: Standard assay procedures and pharmacological techniques were used.

Major Findings: Administration of ethanol (6.0 gm./kg, orally) to rats produced a typical picture of pituitary-adrenal stimulation characterized by decreased levels of adrenal ascorbic acid, increased plasma levels of corticosterone and free fatty acids (FFA), and an increased activity of liver tryptophane peroxidase (TPO). The responses were prevented by hypophysectomy or adrenalectomy, but not by adrenal demedullation.

Pretreatment of animals with dibenamine (10 mg/kg, i.p., 18 hours before ethanol) markedly reduced all of the pituitary-adrenal responses but was almost

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completely ineffective in blocking the deposition of neutral fat in the liver. Chlorisondamine (Ecolid, 1.0 mg/kg, i.p., 30 minutes before ethanol) had no blocking action on the responses of the adrenal ascorbic acid, plasma corticosterone, or liver TPO. In fact the drug intself evoked an acute hypersecretion of ACTH from the pituitary. However, pretreatment with chlorisondamine did block much of the FFA response, and partially prevented the deposition of liver triglyceride produced by ethanol.

Significance to the Program of the Institute: This problem is of interest since it may lead to an understanding of mechanisms involved in derangements of lipid metabolism that lead to hepatic cirrhosis and other diseases.

Proposed Course of Project: Further studies on the role of the pituitary-adrenal system in mobilization and deposition of tryglycerides will be undertaken. Particular attention will be given to studies of the ability of various pharmacological agents to interfere with normal fat mobilization processes.

Part B included: Yes

Serial No. NHI-173
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Brodie, Bernard B., Butler, William M., Jr., Horning, Marjorie G., Maickel, Roger P., and Maling, Harriet M.: Alcohol Induced Triglyceride Deposition in Liver Through Derangement of Fat Transport. "Am. J. of Clin. Nutrition", in press.

Brodie, Bernard B., Maling, Harriet M., Horning, Marjorie, G., and Maickel, Roger P.: Triglyceride Deposition in Rat Liver Through Derangement of Fat Transport by Alcohol and Other Chemical Agents, "Proc. Internat. Sym. on Drugs Affecting Lipid Metab.", Milan, Italy, June 2-4, 1960, in press.

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Serial No. NHI-174
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Penetration of Drugs into Erythrocytes

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mrs. Jean M. Johnson

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.7
Professional: 0.3
Other: 0.7

Project Description:

Objectives: To study the permeability characteristics of cells and to determine which properties of drugs govern their entry into cells.

Methods Employed: Human erythrocytes were suspended in Tyrode's solution (pH 7.4) containing 1 to 4 mmol/l of a drug, and the suspensions shaken in an incubator at 37° C. After various intervals, the cells were isolated by centrifugation and analyzed for the drug.

Major Findings: A number of basic organic compounds penetrated the red cell at rates related to the lipid/water partition coefficient at pH 7.4. For example, compounds with a moderate to high lipid-solubility like antipyrine, aniline, p-toluidine, bufotenine, N-acetyl-4-aminoantipyrine and procaine amide entered the cell at rates too rapid to measure. Drugs of relatively low lipid-solubility like serotonin, epinephrine, Darstine and norepinephrine penetrated the red cell more slowly; and three quaternary ammonium compounds with very low lipid-solubilities, tensilon, tetramethylammonium and procaine amide ethobromide, penetrated hardly at all. These results support the view that the erythrocyte membrane behaves toward foreign organic compounds as a lipid-like layer.

Penetration by quaternary ammonium ions was also investigated by measuring the rates of hemolysis of the cells in isotonic solutions of the drugs. Darstine produced complete hemolysis in 30 minutes, whereas tensilon, procaine amide ethobromide and tetramethylammonium produced almost no hemolysis in 3 hours. Thus two lines of evidence indicate that Darstine, although a quaternary ammonium ion, readily crosses the erythrocyte membrane. Probably, the unusually high lipid-solubility of Darstine enables it to penetrate lipoid areas of the membrane.

Since the unionized form of weak organic electrolytes is usually much more lipid-soluble than the ionized form, drugs would be expected to penetrate a lipoid cell boundary more rapidly at a pH at which they are more unionized. In accord with this view, the weak base serotonin entered the red cell most rapidly at pH 8, less rapidly at pH 7.4, and least rapidly at pH 6.7.

The lipoid membrane thesis also applies to the penetration of organic acids. For example, weak acids with moderate to high lipid-solubilities like phenol, sulfadiazine, p-aminobenzoic acid, sulfathiazole, 5-nitrosalicylic acid and salicylic acid penetrated red cells at rates too rapid to measure. Acids with low lipid-solubilities like phenol red, sulfosalicylic acid, p-aminohippuric acid and sulfanilic acid, entered the cells at considerably slower rates. Although phenol red, sulfanilic acid and sulfosalicylic acid have extremely low lipid-solubilities, they enter the red cell much more rapidly than do quaternary ammonium ions with similar lipid-solubilities. This finding might be explained on the assumption that lipid-insoluble organic anions enter the cell by diffusing through large, positively-charged pores; organic cations would not be able to pass through these pores, and would be excluded from the cell unless they had a great enough lipid-solubility to penetrate the lipoid portion of the membrane.

Significance to bio-medical research and the program of the Institute: Information regarding the factors which govern the penetration of drugs into living cells may lead to a better understanding of the nature of the cell membrane.

Proposed Course of Project: Investigate the penetration of drugs into the cells of various body tissues.

Part B included: Yes

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Individual Project Report
Calendar Year 1960

Part B

Honors and Awards: None

Publications:

Schanker, L.S., Mechanisms of Drug Absorption and Distribution, Annual Review of Pharmacology, in press.

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Serial No. NHI-175
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Factors Influencing Active Transport Processes

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mr. John J. Jeffrey

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.7
Professional: 0.2
Other: 0.5

Project Description:

Objectives: To investigate the effects of hormones, drugs, nutrition, and other factors on the active transport of substances across body membranes.

Methods Employed: The extent of active transfer of uracil across the intestinal epithelium was measured in vitro using sacs of everted small intestine of the rat.

Major Findings: Starvation of rats for 1 to 4 days produces a 160 to 600 per cent increase in the extent to which uracil is actively transported across the intestinal epithelium in vitro. The most marked increases occurred after 2 and 3 days of fasting when the degree of transport was increased by 300 and 550 per cent. After 4 days, uracil transport rose to 600 per cent of the control value and appeared to approach a maximum. The enhancement of active transport disappeared when the starved animals were allowed a normal diet for 3 days.

The possibility that the increased concentration gradients resulted from a decrease in the rate of passive back-diffusion of uracil was ruled out when it was shown that the rate of passive transfer of uracil is increased in starved animals.

Hypophysectomy of rats produces a 250 per cent increase in the extent of active intestinal transfer of uracil. The increase was observed between 8 and 23 days after removal of the pituitary, but was not apparent during the first 3 days after hypophysectomy. Starvation of hypophysectomized rats for 2 days did not result in any additional increase in uracil transport. The rate of passive intestinal transfer of uracil was not altered by hypophysectomy.

The adrenal cortical hormones do not appear to influence the intestinal transport of uracil. For example, administration of ACTH to hypophysectomized rats produced a marked elevation in plasma corticosterone, but had no effect on uracil transport. Cortisone administration to normal rats resulted in a slight depression of uracil transfer, and adrenalectomy of normal rats had no apparent effect on the active transfer mechanism.

Preliminary results indicate that hyperthyroid rats have a lowered ability to actively transport uracil.

The similar effect of starvation and hypophysectomy on the intestinal transfer of uracil suggests that both factors may operate through a common mechanism. The mechanism could involve food intake and nitrogen balance since hypophysectomized rats eat less than normal rats, and they show a decrease in body weight.

Significance to bio-medical research and the program of the Institute: Information regarding the interactions between active transport processes and other physiological processes might lead to a better understanding of how the body regulates the uptake and utilization of vital substrates.

Proposed Course of Project: (1) Further investigation of the increased active intestinal transfer of pyrimidines resulting from starvation and hypophysectomy. (2) Further study of the influence of various hormones on the active transfer of uracil. (3) Investigate the effect of starvation, hypophysectomy, hormones, etc., on other active transport processes.

Part B included: No

Serial No. NHI-176
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Intracellular Localization of Drugs in Erythrocytes

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mrs. Jean M. Johnson

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.4
Professional: 0.1
Other: 0.3

Project Description:

Objectives: To determine: (1) the extent of intracellular localization of various drugs; (2) which components of cells combine with drugs; and (3) which properties of drugs govern their degree of intracellular localization.

Methods Employed: Human erythrocytes were incubated at 37° C with solutions of various drugs in Tyrode's solution. After equilibrium was established, cells were isolated by centrifugation and both cells and supernatant fluid assayed for the drug. The results were expressed as the cell water/extracellular water concentration ratio of the drug.

To estimate the degree of intracellular binding of drugs, the incubated red cells were ruptured by sonic oscillation. Various dilutions of the suspension of broken cells were ultrafiltered through cellophane, the degrees of binding plotted graphically, and the binding for undiluted cells determined by extrapolation of the data. Binding of drugs to ghosts (stromata) of red cells was estimated in a similar manner after incubation of the ghosts with the drug. The degree of hemoglobin binding of drugs was measured by ultrafiltering drug solutions containing various

concentrations of hemoglobin.

Major Findings: The results shown in Table 1 indicate that the extent of intracellular binding of drugs in erythrocytes can be estimated accurately by measuring the binding to suspensions of broken cells. For example, the calculated degrees of intracellular binding of 3 neutral molecules, ethylene glycol, p-nitroaniline, and sulfaguanidine, were 0, 77, and 55% respectively; the measured degrees of binding of these drugs to broken cells were 0, 77, and 52%. Moreover, the calculated binding of an organic anion, phenol red, was 78%, and the measured binding to broken cells was 76%.

For the drugs studied, nearly all of the intracellular binding was accounted for as binding to hemoglobin.

TABLE 1. Binding of Drugs to Red Cells, Hemoglobin, and Red Cell Ghosts

Drug	Cell Water/Extracellular Water Concentration Ratio of Drug	Calculated degree of intracellular binding	Measured Degree of Binding		
			Broken red cells	Hemoglobin	Ghosts
		%	%	%	%
Ethylene glycol	0.98	0	0	-	-
p-Nitroaniline	4.41	77	77	72	<2
Sulfaguanidine	2.22	55	52	40	<2
Phenol red	3.20	78*	76	75	<2

* Calculated on the assumption that phenol red, like other anions, would have a cell water/extracellular water concentration ratio of 0.7 in the absence of intracellular binding.

Significance to bio-medical research and the program of the Institute: Although the intracellular localization of drugs is one of the most important factors in determining the distribution of drugs in the body, almost nothing is known about it. The method of estimating intracellular binding, devised in the present investigation of the red cell, may be applicable to the study of drug localization in other types of body cells.

Proposed Course of Project: Investigate the intracellular localization of drugs in various body tissues.

Part B included: No

Serial No. NHI-177
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Transport of Pyrimidines and Purines Across the Intestinal Epithelium

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mr. John J. Jeffrey

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.7
Professional: 0.2
Other: 0.5

Project Description:

Objectives: To describe the processes by which naturally occurring pyrimidines and purines and structurally related foreign compounds penetrate cell membranes.

Methods Employed: The transfer of substances across the intestinal epithelium was studied in vitro using sacs of everted small intestine.

Major Findings: Uracil is actively transported across the intestinal epithelium of the rat, hamster, and frog. Transport is inhibited markedly by metabolic poisons like iodoacetate, 2,4-dinitrophenol, p-chloromercuribenzoate or phlorizin, and is blocked completely under anaerobic conditions.

The active transport of uracil is strongly inhibited by pyrimidines like thymine (5-methyluracil), 5-aminouracil, 5-bromouracil, 5-fluorouracil, 2-amino-4,6-dihydroxypyrimidine, and 4-amino-2,6-dihydroxypyrimidine. Cytosine, 6-azauracil and 6-azathymine produce a smaller degree of inhibition; and barbituric acid and barbital have no effect on the transport of uracil. The results suggest that the hydroxyl group at the 6-position of the pyrimidine ring is required for interaction with the pyrimidine transport system. In contrast, the nature of the substituent at

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Individual Project Report
Calendar Year 1960

Part B

Honors and Awards: None

Publications:

Schanker, L.S. and Tocco, D.J., Active Transport of Some Pyrimidines Across the Rat Intestinal Epithelium. J. Pharmacol. and Exper. Therap., 128: 115-121, 1960

Schanker, L.S. and Jeffrey, J.J., Active Transport of Foreign Pyrimidines Across the Intestinal Epithelium. Nature, in press.

Schanker, L.S., On the Mechanism of Absorption of Drugs from the Gastrointestinal Tract. J. Med. and Pharm. Chem., 2: 343-359, 1960.

the 2- or 5-position of the uracil molecule is relatively unimportant in this respect.

Two foreign pyrimidines used as anti-tumor agents, 5-fluorouracil and 5-bromouracil, are actively transported across the rat intestinal epithelium. These drugs are transported against a concentration gradient, their transport process becomes saturated at high concentrations of the drugs, and the compounds compete with uracil for the active transfer process.

The purine compounds, hypoxanthine, xanthine and uric acid, depress the active intestinal transport of uracil. Uric acid is the weakest inhibitor, and hypoxanthine the strongest, producing a degree of inhibition greater than that of some pyrimidines. Like the pyrimidines that have an affinity for the transport process, these purines have the essential 6-hydroxy group in the pyrimidine portion of their molecule.

Despite the apparent interaction of hypoxanthine, xanthine and uric acid with the pyrimidine transfer process, these purines do not appear to be actively transported across the intestinal epithelium.

Significance to bio-medical research and the program of the Institute: Knowledge of the mechanisms by which natural substrates traverse the cell boundary should increase our understanding of the functions of the cell and its components.

Proposed Course of Project: (1) Further investigation of the interaction of purines with the pyrimidine transport system of the small intestine. (2) Search for possible intermediates formed during the active transport of pyrimidines. (3) Investigation of the passage of purines and pyrimidines across the boundaries of other cells.

Part B included: Yes

Serial No. NHI-178
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individaul Project Report
Calendar Year 1960

Part A

Project Title: Distribution and Fate of Substances Injected into the Cerebrospinal Fluid

Principal Investigators: Dr. Leon D. Prockop
Dr. Lewis S. Schanker

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.6
Professional: 0.6
Other: 0

Project Description:

Objectives: To develop procedures for studying the distribution of substances within the central nervous system and the exit of substances from the cerebrospinal fluid (CSF) after injection into a lateral ventricle. Results from these studies should give information about the nature of various membranes within the central nervous system.

Methods Employed: Using a stereotaxic instrument, a technique was developed by which micro volumes of fluid can be injected into a lateral ventricle of the rabbit brain.

Major Findings: Inulin-C^{14}, injected into a lateral ventricle, appeared in the cisterna magna within 5 minutes; about 80% had left the ventricle in 1 hour. Only traces of inulin were detected in the opposite lateral ventricle after an hour.

Preliminary results indicate that traces of inulin penetrate the ependymal lining of the ventricle. For example, 1 hour after intraventricular injection, inulin was detected in the 0.5 mm section of brain tissue underlying the first 0.5 mm section which

included the ependyma.

Labeled inulin, sucrose and mannitol appeared in the urine in significant amounts after intraventricular injection. The fraction of the injected dose recovered in urine after six hours was: inulin, 52%; sucrose, 38%; and mannitol, 23%. The rates of urinary excretion of the substances after intravenous administration ranked in the same order as that observed after injection into CSF, inulin > sucrose > mannitol, indicating that the rates of exit from CSF may be similar for the 3 compounds.

In any case, the ready exit of large, lipid-insoluble molecules from cerebrospinal fluid suggests a rapid filtration of fluid from CSF to blood across a highly porous boundary.

Significance to bio-medical research and the program of the Institute: Knowledge of the distribution and fate of substances injected into the cerebrospinal fluid would help to clarify a large amount of pharmacologic data previously obtained by injecting drugs by this route.

Proposed Course of Project: (1) Investigate the distribution of drugs within the cerebrospinal fluid and the brain after intraventricular, intracisternal, and intracerebral injection. (2) Study the transfer of drugs from cerebrospinal fluid into blood.

Part B included: Yes

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Individual Project Report
Calendar Year 1960

Part B

Honors and Awards: None

Publications:

Brodie, B.B., Kurz, H., and Schanker, L.S., The Importance of Dissociation Constant and Lipid-solubility in Influencing the Passage of Drugs into the Cerebrospinal Fluid. J. Pharmacol. and Exper. Therap., 130: 20-25, 1960.

Serial No. NHI-179
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Secretion of Substances into Bile

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mr. Panayotis A. Nafpliotis

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.15
Professional: 0.05
Other: 0.1

Project Description:

Objectives: To describe the processes whereby drugs and other substances pass from blood into bile.

Methods Employed: Anesthetized rats with ligated renal pedicles and cannulated bile ducts received an intravenous injection of a drug. Bile and plasma were assayed for the drug after various times.

Major Findings: Previous work has shown that large, lipid-insoluble molecules (saccharides) enter bile at rates roughly related to their molecular weight -- the larger the molecule, the slower the rate. The present study concerns the biliary excretion of quaternary ammonium compounds.

Procaine amide ethobromide appears in bile in a concentration about sixty times that in plasma. About half of the drug in bile is in a combined form, possibly a glucuronide.

Decamethonium-N-methyl-C^{14} is excreted in bile in a concentration similar to or slightly less than that in plasma.

The liver is known to secrete certain organic anions (sulfonic acids) in very high concentrations. The present results show that the liver also actively secretes certain organic cations.

Significance to bio-medical research and the program of the Institute: This study may lead to a better understanding of the permeability of hepatic cells and the means by which drugs and their metabolic products pass into bile.

Proposed Course of Project: Further investigation of the biliary excretion of drugs.

Part B included: No

Serial No. NHI-180
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Binding of Drugs to Plasma Proteins

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mr. Panayotis A. Nafpliotis

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.25
Professional: 0.05
Other: 0.20

Project Description:

Objectives: To determine which physical properties of drugs are important in governing their binding to plasma proteins. To study the nature of the binding process.

Methods Employed: The binding of drugs to human serum albumin and human gamma globulin (fraction II) was measured by ultrafiltration through cellophane. The concentration of the drugs was 0.5 mM, that of albumin 3 per cent and that of globulin 6 per cent; the substances were dissolved in 0.1 M Na_2HPO_4 solution, pH 7.4.

The binding of drugs to charcoal was measured by shaking 5 mg of Nuchar-C190-N charcoal with 25 ml of a 0.5 mM solution of drug in 0.1 M Na_2HPO_4 solution, pH 7.4. After shaking, the mixture was centrifuged, and the supernatant fluid assayed for the drug.

Major Findings: The initial phase of this project has been an investigation of the protein binding of undissociated molecules. The compounds studied were mainly derivatives of aniline or phenol, which were more than 95% undissociated at the pH of the test solutions.

Binding to serum albumin of 19 compounds ranged from zero to 68 per cent. The degrees of globulin binding paralleled roughly, but were much lower than, the degrees of albumin binding.

TABLE 1. Binding of Some Aniline Derivatives to Serum Albumin, Serum Globulin, and Charcoal.

Drug	PER CENT BINDING		
	3% Albumin Solution	6% Globulin Solution	Charcoal
p-Nitroaniline	48	18	63
m-Nitroaniline	38	18	63
p-Aminophenol	37	-	59
Aniline	15	7	39
o-Aminophenol	14	-	36
Sulfaguanidine	11	8	31
Sulfanilamide	6	7	36
m-Aminophenol	6	-	33

A rough relation between protein binding and charcoal adsorption of a number of aniline derivatives is apparent from the data in Table 1; in general, the greater the protein binding, the greater was the adsorption to charcoal. The results suggest that the interaction between these molecules and protein does not involve specific chemical groups; rather, binding may depend largely on surface Van der Waals forces.

Significance to bio-medical research and the program of the Institute: This study may lead to a better understanding of the principles governing the binding of drugs to plasma proteins. The plasma binding of drugs is an important factor in regulating the entry of drugs into the central nervous system and into tissue cells in general.

Proposed Course of Project: Investigate the plasma protein binding of drugs with widely different physico-chemical properties in an attempt to define the nature of the binding processes.

Part B included: No

Serial No. NHI-181
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Factors Controlling Drug Metabolism

Principal Investigators: Dr. Allan Conney
Dr. J. J. Burns

Other Investigators: Dr. I. A. Michaelson
Dr. Peter Dayton
Miss Ann Murray

Cooperating Units: New York University Research Service, Goldwater Memorial Hospital, New York

Man Years (calendar year 1960):
Total: 3.2
Professional: 2.2
Other: 1

Project description:

Objectives: To investigate the various factors which may influence drug metabolism such as administration of other drugs, thyroid hormone, ascorbic acid deficiency, and liver disease.

Patient Material: None

Major Findings:

(a) Pretreatment of rats with phenobarbital, phenylbutazone, chlorcyclizine, and aminopyrine increases the activity of liver microsomal enzymes that metabolize zoxazolamine, phenylbutazone, hexobarbital, aminopyrine, and various other drugs. The increases in enzyme activity are paralleled by accelerated drug metabolism in the intact animal and by a shortened duration of drug action. Recent results also indicate that phenylbutazone administration speeds up the metabolism of aminopyrine in human subjects receiving the drug. In addition, evidence has been obtained that administration of barbiturates to man may speed up the metabolism of certain coumarin anticoagulants.

(b) Administration of thyroxin to rats can either shorten the duration of action of zoxazolamine by accelerating its metabolism or prolong the duration of action of hexobarbital by inhibiting its metabolism. Administration of the hormone does not alter the activity of the zoxazolamine-metabolizing enzyme system in liver microsomes but does decrease the activity of the hexobarbital-metabolizing enzyme system in liver microsomes. These results are of interest in view of the many reports indicating that patients with thyrotoxicosis differ from normal individuals in their response to drugs.

(c) Some of the symptoms of scurvy in guinea pigs have been shown to be reversed by the administration of 3-methylcholanthrene. These include a marked reduction of the incidence of hemorrhage, reduction of bone resorption, preservation of dentinogenesis, and prevention of death of odontoblasts. The effect 3-methylcholanthrene exerts upon scurvy does not appear to be mediated by the induction of ascorbic acid synthesis nor by an increased retention of the vitamin.

Significance to the Program of the Institute: Ascorbic acid is necessary for the maintenance of normal connective tissue which is important for the integrity of the cardiovascular system.

Proposed Course of Project: Project is terminated.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Conney, A. H., Davison, C., Gastel, R. and Burns, J. J.: Adaptive Increases in Drug-Metabolizing Enzymes Induced by Phenobarbital and Other Drugs. J. Pharm. and Exptl. Therap., 130: 1-8, Sept., 1960.

Conney, A. H. and Garren, L.: Contrasting Effects of Thyroxin on Zoxazolamine and Hexobarbital Metabolism. Biochem. Pharm., in press.

Brodie, B. B., Burns, J. J. and Weiner, M.: Metabolism of Drugs in Subjects with Laennec's Cirrhosis. Medicina Experimentalis, 1: 290-292, 1959.

Burns, J. J. and Shore, P. A.: Biochemical Effects of Drugs. Ann. Rev. Pharmacology, Vol. I, in press.

Conney, A. H. and Burns, J. J.: Biochemical Pharmacological Considerations of Zoxazolamine and chlorzoxazone Metabolism. Annals of the New York Academy of Sciences, 86: 167-177, 1960.

Conney, A. H., Michaelson, I. A. and Burns, J. J.: Stimulatory Effect of Chlorcyclizine on Barbiturate Metabolism, J. Pharm. and Exptl. Therap., in press.

Chenkin, T., Dayton, P. G., Weisberg, L. and Weiner, M.: Effect of Starvation, Acenocoumarin and Vitamin K on Coagulation Patterns of Guinea Pigs. Experimental Medicine and Surgery, 17: 219, 1960.

Mark, L. C. Brand, L., Dayton, P. G., Taller, D. and Papper, E. M.: Passage of Barbital into the Cerebral Spinal Fluid. Proc. Soc. Exp. Biol. and Med., 103: 606, 1960.

Brand, L., Mark, L. C., Burns, J. J., Dayton, P. G., Taller, D. and Papper, E. M.: Physiological Disposition of Methitural in Man. Medicina Experimentalis, 1: 339, 1959.

Part I. [illegible]

[illegible]

[illegible]

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Serial No. NHI-182
1. Chemical Pharm.
2. Clinical Pharm.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies with Ascorbic Acid

Principal Investigators: Dr. J. Burns
Dr. A. H. Conney

Other Investigators: Dr. P. G. Dayton
Dr. George Bray
Mr. Julian Kanfer
Miss Carole Evans
Miss Ruth Gastel

Cooperating Units: New York University Research Service,
Goldwater Memorial Hospital, New York

Man Years (calendar year 1960):
Total: 5.4
Professional: 2.4
Other: 3.0

Project Description:

Objectives: (1) To determine the mechanisms required for the formation and degradation of L-ascorbic acid. (2) To investigate why drugs stimulate the formation of ascorbic acid. (3) To gain information on the functions of ascorbic acid.

Major Findings:

(a) Various drugs stimulate the synthesis of L-ascorbic acid from glucose or galactose in rats. Although the specific mechanism is not known, drugs appear to stimulate the activity of a key enzyme required in these reactions, UDPG dehydrogenase. 3-Methylcholanthrene has been found to be the most potent compound which stimulates ascorbic acid biosynthesis in rats. Turnover rate studies with L-ascorbic acid-1-C^{14} have been carried out, and they further characterized the potent effect of this hydrocarbon not only on the formation of the vitamin but also on its breakdown.

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(b) Two pentonic acids have been identified as products of L-ascorbic acid metabolism in rats. It has now been found that a 5-carbon ascorbic acid analog, L-erythroascorbate, is formed from L-xylonic acid by enzymes in rat liver. These results suggest a new cyclic mechanism of vitamin C metabolism.

(c) Zoxazolamine was found to have a more prolonged muscular relaxing action in ascorbic acid-deficient guinea pigs than in normal guinea pigs. This has been shown to result from decreased activity of the zoxazolamine-metabolizing enzyme in the liver microsomes of the deficient animals. It should be noted that various other reports have suggested a role for ascorbic acid in drug metabolism.

(d) It has been commonly thought that patients with liver disease metabolize drugs at a slower rate than healthy individuals. However, it has now been found that certain representative drugs (salicylate, phenylbutazone, Dicumarol, and aminopyrine) are metabolized at a normal rate in patients with Laennec's cirrhosis. Although patients with liver disease are in many instances more sensitive to the action of a drug, this doesn't necessarily reflect an altered rate of the drug's metabolic destruction.

Significance to the Program of the Institute: Studies on induced enzyme synthesis will give information on those factors which regulate the duration of drug action.

Proposed Course of Project: This project has been terminated.

Part B included: Yes

Serial No. NHI-182
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Burns, J. J. and Ashwell, G.: L-Ascorbic Acid, The Enzymes, 3:387-406, 1960.

Burns, J. J.: Vitamin C Activity of D-Ascorbic Ácid. Vitamin Metabolism. Proc. of the IV International Congress of Biochemistry, Vienna, 1958, Ed. by W. Umbreit and H. Molitor, Pergamon Press, New York, 1960, p. 313-315.

Burns, J. J.: Ascorbic Acid, Chemical Pathways of Metabolism, 1: 341-356, 1960.

Burns, J. J. and Conney, A. H.: Water-Soluble Vitamins (Ascorbic Acid, Nicotinic Acid, Vitamin B_6, Biotin, Inositol). Annual Rev. of Biochem., 29: 413-436, 1960.

Burns, J. J., Conney, A. H., Dayton, P. G., Evans, C., Martin, G. R. and Taller, D.: Observations on the Drug Induced Synthesis of D-Glucuronic, L-Gulonic and L-Ascorbic Acid in Rats. J. Pharm. and Exptl. Therap., 129: 132-138, 1960.

Evans, C., Conney, A. H., Trousof, N. and Burns, J. J.: Metabolism of D-Galactose to D-Glucuronic Acid, L-Gulonic Acid and L-Ascorbic Acid in Normal and Barbital-Treated Rats. Biochim. et Biophys. Acta, 41: 9-14, 1960.

Kanfer, J., Ashwell, G. and Burns, J. J.: Formation of L-Lyxonic Acid and L-Xylonic Acid from L-Ascorbic Acid in Rat Kidney. J. Biol. Chem. 235: 2518-2521, Sept. 1960.

Martin, C. R., Fullmer, H. M. and Burns, J. J.: Observations on the Effect of 3-Methylcholanthrene in Scorbutic Guinea Pigs. Proc. Soc. Exptl. Biol. and Med., in press.

Conney, A. H., Bray, G., Evans, C. and Burns, J. J. Interrelationships Between Metabolism of Ascorbic Acid and Drugs. Annals N. Y. Acad. of Sci., in press.

Ashwell, G., Kanfer, J., Smiley, J. D. and Burns, J. J. Metabolism of Ascorbic Acid and Related Uronic Acids, Aldonic Acids and Pentoses. Annals N. Y. Acad. of Sci., in press.

Fullmer, H., Martin, G. and Burns, J. J.: Vitamin C and the Maintenance of Normal Dental Structures. Annals N. Y. Acad. of Sci., in press.

Perel, J. M. and Dayton, P. G.: A Convenient Synthesis of Crystalline L-Threonolactone. J. Org. Chem. 25: 2044, 1960.

Dayton, Peter G. and Weiner, Murray. Ascorbic Acid and Blood Coagulation. Annals N. Y. Acad. of Sci., in press.

Serial No. NHI-183
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on New Drugs for Arthritis and Gout

Principal Investigators: Dr. J. J. Burns
Dr. Peter G. Dayton

Other Investigators: None

Cooperating Units: New York University Research Service at Goldwater Memorial Hospital, Department of Medicine, Mount Sinai Hospital, New York, and Geigy Laboratories, Basel, Switzerland

Man Years: (Calendar Year 1960)
Total: .66
Professional: .66
Other: None

Project Description:

Objectives: To study the physiological disposition and metabolism of phenylbutazone analogs in order to find more useful drugs of this type for the treatment of arthritis and gout.

Major Findings:

(a) Renal clearance studies, in man and dog, were made with series of phenylbutazone analogs, related in structure and similar in lipid solubility and binding to plasma protein (about 98% in man) but differing widely in acidity (range of pKa, 2.3 to 5.5). It was found that the rate of renal excretion of these compounds varied inversely with their pKa. The more acidic analogs were rapidly excreted in man under the normal circumstances of acid urine (net secretion in clearance studies), the less acidic analogs were very slowly excreted (net reabsorption). Stop-flow studies in the dog revealed that peak secretion of the drugs, when demonstrable, occurred in the proximal tubular segment.

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Reabsorption occurred chiefly from the acidified urine of the more distal tubular segments, but the less acidic analogs may be appreciably reabsorbed more proximally also.

Appreciation of such structure-excretion relationships have proven helpful in anticipating the rate of renal excretion and the plasma half-life of newly synthesized drugs in this series.

(b) The conversion of a thio analog of phenylbutazone (G-25671) to sulfinpyrazone and the conversion of sulfinpyrazone to hydroxy-sulfinpyrazone has been shown to occur in man. The metabolism of sulfinpyrazone to hydroxy-sulfinpyrazone is analogous to the metabolism of phenylbutazone to a product formed by the introduction of a hydroxyl group in the para position of the benzene ring. Optically active sulfinpyrazone was isolated from urine of human subjects who had received G-25671. Comparison with synthetic d-sulfinpyrazone indicated that the material isolated from urine had only about 10% of the optical activity of the pure d-isomer. During the oxidation of the thio ether of G-25671 it appears that both isomers are formed but in different proportions. It is of interest that the hydroxylation of phenylbutazone on the β carbon of the side chain also gives rise to an optically active metabolite.

Both sulfinpyrazone and its hydroxy metabolite are potent uricosuric agents in man. Intravenous doses of about 50 mg of each can double the renal clearance of uric acid.

(c) Oxyphenbutazone, a para hydroxy metabolite of phenylbutazone has a pKa of 4.6 and is slowly and completely metabolized in man (half-life 3 days). Introduction of a keto group into the α position of the butyl side chain yields a strongly acidic compound (pKa 2.2) which is rapidly excreted in urine (half-life 6 hours). On the other hand, replacement of the normal butyl side chain of oxyphenbutazone by a tertiary butyl group leads to a weakly acidic compound (pKa 7.0) which is rapidly and completely metabolized (half-life 1 hour). Evidence for excretion of drug glucuronides was obtained only after administration of the tertiary butyl analog. Thus, relatively minor alterations in the chemical structure of oxyphenbutazone produce striking changes in acidity which markedly affect the metabolism and excretion of the resulting compounds.

(d) In our previous report we pointed out that several new drugs have resulted from these studies. One of these is oxyphenbutazone, which has been introduced recently in Europe as a new antirheumatic agent under the name of Tenderil. It is expected that this drug will be introduced in the United States in the near future. Two other drugs, sulfinpyrazone (Anturane) and zoxazolamine (Flexin) are used as uricocuric agents for the treatment of chronic gout. Many reports have appeared during the past year which have confirmed our initial observation on the potent uricosuric effect of these agents.

Proposed Course of Project: Eight new analogs of phenylbutazone are available for study in order to further document the structural-activity relationships already observed in this series. Experiments are planned to investigate the importance of biliary excretion in the phenylbutazone series.

In the search for a long-acting uricosuric agent, a methyl-sulfone derivative of phenylbutazone is under clinical trial by Drs. Gutman and Yü at Mount Sinai Hospital, New York.

Significance to the Program of the Institute: A non-toxic potent antirheumatic drug would be of considerable value in the treatment of rheumatic fever. Studies on how drugs effect uric acid excretion increase our general knowledge on the mechanisms by which various naturally occurring compounds are excreted by the kidney.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Conney, A. H., Trousof, N. and Burns, J. J.: The Metabolic Fate of Zoxazolamine (Flexin) in Man. J. Pharm. and Exptl. Therap., 128: 333-339, 1960.

Conney, A. H. and Burns, J. J.: Physiological Disposition and Metabolic Fate of Chlorzoxazone (Paraflex) in Man. J. Pharm. and Exptl. Therap., 128: 340-343, 1960.

Burns, J. J., Yü, T. F., Dayton, P. G., Gutman, A. B. and Brodie, B. B.: Biochemical Pharmacological Considerations of Phenylbutazone and its Analogues. Annals of the New York Academy of Sciences, 86: 253-262, 1960.

Gutman, A. B., Dayton, P. G., Yü, T. F., Berger, L., Chen, W., Sicam, L. F. and Burns, J. J.: A Study of the Inverse Relationship Between pKa and Rate of Renal Excretion of Phenylbutazone Analogs in Man and Dog. Amer. J. Med., in press.

Seegmiller, J. E., Dayton, P. G. and Burns, J. J.: Physiological Disposition and Uricosuric Effect of a Keto Analogue of Oxyphenbutazone (G-29701). Arthritis and Rheumatism, in press.

Burns, J. J., Yü, T. F., Dayton, P. G., Brodie, B. B. and Gutman, A. B.: Search for New Drugs for Arthritis and Gout in the Phenylbutazone Series. Proceedings of the Fourth European Rheumatological Congress, Istanbul, September, 1959, in press.

Serial No. NHI-184
1. Chemical Pharmacology
2. Drug Enzyme Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Mechanism of TPNH-Dependent Enzyme Systems in Liver Microsomes

Principal Investigator: Dr. James R. Gillette

Other Investigator: Mr. Jerome J. Kamm

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: 0.7
Professional: 0.2
Other: 0.5

Project Description:

Objectives: To elucidate the mechanisms of the microsomal enzyme systems requiring TPNH. The systems comprise those that catalyze the oxidation of many drugs and other foreign compounds, the formation of cholesterol and steroid hormones, and the reduction of azo, nitro, and tetrazolium compounds.

Major Findings: 1) Oxidative microsomal enzyme systems:

In previous work it was shown that TPNH is oxidized by liver microsomes to yield hydrogen peroxide, and that 4,4'-diaminodiphenyl sulfide (DDS) causes a decrease in the formation of hydrogen peroxide equivalent to the amount of 4,4'-diaminodiphenyl sulfoxide (DDSO) formed. These results suggested the following mechanism:

1. TPNH + O_2 + microsomes $\longrightarrow$ "Hydroxyl-donor" + TPN
2. "Hydroxyl-donor" $\longrightarrow$ H_2O_2
3. "Hydroxyl-donor" + drug substrate $\longrightarrow$ hydroxylated substrate

If the same "hydroxyl-donor" were utilized for the formation of peroxide and the oxidation of the drug substrate, then the Km-TPNH should be the same for both reactions. We found that

the Km-TPNH for the oxidation of DDS to DDSO (6×10^{-5}) is about 6 times larger than that for the formation of peroxide (1×10^{-5} M). The mechanism, therefore, is more complicated than the one previously proposed.

In preliminary experiments we have studied the effect of various concentrations of TPNH on the Michaelis constant for the monomethyl-4-amino-antipyrine (MMAP)-N-dealkylase complex and have found that it changed only slightly; for example, when the concentration of TPNH was increased from 3×10^{-5} M to 3×10^{-4} M, the Km-MMAP was decreased from 1.77 to 1.39×10^{-3} M. Similarly, when the concentration of MMAP was increased from $2\text{-}32 \times 10^{-4}$ M, the Km-TPNH decreased from $16\text{-}5 \times 10^{-6}$ M. If these apparent changes in the Michaelis constants are real, they will be valuable aids in helping us elucidate the mechanisms of these reactions.

2) Reductive microsomal enzyme systems:

a) Reduction of nitro benzene.

Nitrosobenzene and phenyl hydroxylamine, which are probably intermediates in the reduction of nitrobenzene to aniline, were synthesized and used as substrates for microsomal nitroreductase. These compounds were metabolized even more rapidly than nitrobenzene, and therefore do not accumulate during the reduction of the nitro compound. The kinetics of the reduction of nitrobenzene by microsomal nitroreductase were studied by varying the concentrations of both the TPNH and nitrobenzene. Using the data from this experiment, we were able to calculate the constants in the following kinetic formula of Dalziel:

$$\frac{1}{v} = \frac{\emptyset o}{e} + \frac{\emptyset 1}{e\,[s1]} + \frac{\emptyset 2}{e\,[s2]} + \frac{\emptyset 12}{e\,[s1]\,[s2]}$$

where v = velocity of the reaction, e = enzyme concentration, [s1] = concentration of TPN, [s2] = concentration of nitrobenzene.

We found that both $\emptyset o/e$ and $\emptyset 12/e$ were zero, which indicates that no enzyme-substrate complex is formed. These data are consistent with the following mechanism:

1. $E + TPNH \longrightarrow E' + TPN$

2. $E' +$ nitrobenzene $\longrightarrow$ nitrosobenzene $+ E$

b) Reduction of tetrazolium compounds:

Triphenyl tetrazolium (TTC) is reduced by liver microsomes in the presence of TPNH. TTC does not inhibit the reduction of p-nitrobenzoic acid or neoprontosil, but does inhibit the N-dealkylation of MMAP and ethylaniline. It would appear, therefore, that TTC reductase is a different enzyme from either nitro or azo reductase but may be a component of the

oxidative microsomal enzyme systems.

Significance to the Program of the Institute: These studies provide a better understanding of the metabolism of many drugs.

Proposed Course of Project: Attempts will be made to solubilize and purify the microsomal oxidative and reductive enzyme systems. Studies will be made with these purified preparations to determine whether microsomal TPNH oxidase is a component of any of these systems.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Gillette, J. R., Kamm, J. J. and Brodie, B. B.: The Enzymatic Formation of Sulfoxides: The Oxidation of Chlorpromazine and 4,4'-Deaminodiphenyl Sulfide to Sulfoxides by Guinea Pig Liver Microsomes. J. Pharmacol. Expt. Therap., 130, 262, 1960.

Gillette, J. R. and Brodie, B. B.: Oxidation of Drugs and Other Foreign Compounds by Enzyme Systems in liver microsomes. Proceedings of "Celebrasione Spallanzaniana," Pavia, Italy, in press.

Serial No. NHI-185
1. Chemical Pharmacology
2. Drug Enzyme Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Development of Methods for the Assay of Micro Amounts of Chlorpromazine and Other Phenothiazines

Principal Investigators: Dr. James R. Gillette
Dr. William A. M. Duncan

Other Investigators: None

Cooperating Units: Laboratory of Physical Biology,
Dr. Edwin Becker
Dr. Duncan was a guest worker from the Imperial Chemical Industries Ltd.

Man Years (Calendar Year 1960):

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

Objectives: A number of methods have been proposed for the assay of chlorpromazine and other phenothiazines in tissues and biological fluids. However, none of these is sensitive enough to detect the small amount of these phenothiazines present in the plasma of patients.

Major Findings: Nitrous acid and other oxidizing agents such as ceric sulfate, ferric chloride and hydrogen peroxide oxidize chlorpromazine to a red-colored intermediate, which decomposes to chlorpromazine sulfoxide by a second order reaction. The rate of decomposition is decreased by a large excess of chlorpromazine and by concentrated inorganic acids; it is increased by bases and acetate ions.

A new and sensitive photofluorometric method based on these observations has been developed for the assay of chlorpromazine in tissues and biological fluids. Chlorpromazine is extracted from alkalinized (pH 11) biological fluids into heptane containing 2% isoamyl alcohol, and returned to an aqueous phase by shaking the heptane extract with 0.01 N HCl. The fluorescence of the acid extract is measured at 380 μ (activated at 340 μ);

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this fluorescence is due to the blank and chlorpromazine sulfoxide (Davidson et al., J. Pharmacol. Exptl. Therap. 121, 8, 1957). A drop of 1% $NaNO_2$ is added to oxidize chlorpromazine and monomethyl norchlorpromazine to their sulfoxides, and the fluorescence is measured again at 380 mμ. As little as 0.5 μg of chlorpromazine or monomethyl norchlorpromazine per ml of acid extract is measured by this method. Since almost all of the fluorescence of the blank is due to the reagents and not to the plasma, very low plasma concentrations of chlorpromazine can be measured by using large volumes of plasma.

Attempts have been made to identify the red intermediate formed during the oxidation of chlorpromazine. Although Dusinsky et al. have suggested that the red intermediate is a free radical, they have presented no direct evidence to support this view. Our electron magnetic resonance (EMR) studies have indicated that a free radical, which decays by a second order reaction, is formed during the oxidation of chlorpromazine by either ceric sulfate or a hydrogen peroxide-peroxidase system. We have attempted to measure the concentration of the free radical and to relate it to the concentration of the red colored substance(s), but have not been successful since the EMR signals are assymetrical.

Significance to the Program of the Institute: A method is now available for the assay of micro amounts of chlorpromazine in plasma. This method provides a new tool for the study of the physiological disposition of chlorpromazine.

Proposed Course of Project: Studies to determine the mechanism of the ceric sulfate system will be continued.

Part B included: No

Serial No. NHI-186
1. Chemical Pharmacology
2. Drug Enzyme Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Inhibition of Microsomal Enzyme Systems by Triparanol (MER-29)

Principal Investigator: Dr. James R. Gillette

Other Investigators: Mrs. Lillemor Davenport
Mr. Jerome J. Kamm

Cooperating Units: Mrs. Davenport is a Fellow from George Washington University

Man Years: (Calendar Year 1960)
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: Steinberg and co-workers found that triparanol blocks the synthesis of cholesterol by inhibiting the reduction of the 24, 25 double bond of desmosterol. The aim of this study is to learn if triparanol inhibits other microsomal enzyme systems of liver.

Major Findings: The effect of triparanol on the metabolism of a number of drugs by a 9,000 supernatant system of liver was studied at two concentrations of the inhibitor, 2×10^{-4} M and 1×10^{-3} M. Reactions inhibited more than 50% by 1×10^{-3} M were the N-dealkylation of monomethyl-4-aminoantipyrine, N-methylaniline, and chlorpromazine, the O-dealkylation of acetanisidine, the sidechain oxidation of hexobarbital and the hydrolysis of procaine by liver esterase; those inhibited less than 50% were the N-dealkylation of ethylaniline, imipramine, methamphetamine, ephedrine, benadryl and the hydrolysis of procaine by plasma esterase (human). At $2\text{-}4 \times 10^{-4}$ M concentrations of triparanol, only the N-dealkylation of monomethyl-4-aminoantipyrine, the O-dealkylation of acetanisidine, the sidechain oxidation of hexobarbital and the hydrolysis of procaine by liver were inhibited more than 15 percent. The reduction of p-nitrobenzoic acid and prontosil was not inhibited with 5×10^{-4} M triparanol.

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It has been difficult to demonstrate that triparanol inhibits hexobarbital metabolism, in vivo. Preinjection i.p. with 100 mg/kg of the inhibitor 15 hours before hexobarbital prolongs the sleeping time of mice about 30% ($p = < 0.05$). But prolongation in sleeping time was not significant when a 100 mg/kg dose was preinjected 30 minutes before the hexobarbital ($p = 0.1 - 0.05$). The lack of effect of triparanol in 30 minutes is probably due to its precipitation in and slow absorption from the peritoneal cavity.

Significance to the Program of the Institute: Since triparanol is being used in long term therapy to lower the plasma level of cholesterol in patients, it is important that we know its effects on other enzyme systems.

Proposed Course of Project: We will study the effect of prolonged administration of triparanol on the microsomal enzyme systems.

Part B Included: No

Serial No. NHI-187
1. Chemical Pharmacology
2. Drug Enzyme Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Mechanism of Induction of Liver Microsomal Enzymes by Foreign Compounds

Principal Investigator: Dr. James R. Gillette

Other Investigators: Mrs. Lillemor Davenport
Mr. James V. Dingell

Cooperating Unit: Mrs. Davenport is a Fellow from George Washington University

Man Years (calendar Year 1960):
Total: 0.5
Professional: 0.1
Other: 0.4

Project Description:

Objectives: The preinjection of certain foreign compounds causes an increase in the activity of the enzyme systems that metabolize drugs. The object of these studies is to discover the importance of factors, such as species, sex, age, nutritional state, hormonal balance, drug dose, duration of drug treatment, that can alter or prevent this increase.

Major Findings: Preinjection of imipramine in rats increased the activity of enzymes that metabolize impiramine, monomethyl-4-aminoantipyrine and acetanilide (9000 x g liver supernatant) Preinjection of chlorpromazine did not alter the metabolism of either monomethyl-4-aminoantipyrine or imipramine. Since both chlorpromazine and imipramine have a propyl dimethyl amino sidechain that undergoes dealkylation, the difference in the effect of these compounds is puzzling.

A series of preliminary experiments have provided evidence which suggests the following conclusions: 1) Starvation does not prevent the inductive effect of benzpyrine. 2) The inductive effect of methylcholanthrene is not altered by dibenamine, an adrenergic blocking agent that reduces the ethanol-induced accumulation of neutral fat in rat liver. 3) Reduction of

thyroid activity in rats does not prevent the inductive effect of phenobarbital and methylcholanthrene. 4) The activities of microsomal enzyme systems are increased by drugs to a less degree in guinea pigs than in rats; in adult guinea pigs phenobarbital does not increase the metabolism of either acetanilide or monomethyl-4-aminoantipyrine.

Significance to the Program of the Institute: Studies on the mechanism of induction of the microsomal enzyme systems should provide a better understanding of the variation among patients to metabolize drugs. In view of the use of a number of drugs over prolonged periods of treatment, the possible effect of these drugs on the microsomal enzyme system may be quite important.

Proposed Course of Project: The survey of possible factors that could alter the induction of the microsomal enzyme systems will be continued.

Part B Included: No

Serial No. NHI-188
1. Chemical Pharmacology
2. Drug Enzyme Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Physiological Distribution and Metabolism of Imipramine (Tofranil), A Drug Used in Treatment of Psychiatric Depression

Principal Investigator: Dr. James R. Gillette

Other Investigators: Mr. James V. Dingell

Cooperating Units: None

Man Years (Calendar Year 1960):
Total: 0.6
Professional: 0.1
Other 0.5

Project Description:

Objectives: To study the plasma disappearance, tissue distribution, and metabolism of imipramine.

Methods Employed: A sensitive method for the assay of imipramine has been developed. Imipramine is extracted from alkalinized aqueous solutions into heptane. The heptane extract is shaken with 0.2 M phosphate buffer, pH 6.0, to remove monomethyl norimipramine and hydroxy-imipramine, and then with 0.1 N HCl to remove imipramine. An aliquot of the acidic phase is made alkaline and the fluorescence activated at 290 mμ is measured at 410 mμ. Fluorescence is proportional to imipramine in concentrations of 0.4-10 γ/ml.

Major Findings: It was shown previously that imipramine administered i.v. to rabbits rapidly leaves plasma and accumulates in tissues. It was also shown that little imipramine can be isolated from the tissues or urine 24 hours after administration; the drug, therefore, is extensively metabolized. Herrman et al. have isolated and identified a number of the metabolites from the urine of rabbits. Their results indicate that imipramine undergoes both hydroxylation and N-dealkylation to yield monomethyl norimipramine, norimipramine, hydroxy-imipramine and its glucuronide, and hydroxy-monomethyl norimipramine and its glucuronide.

We have found that imipramine is oxidized by a TPNH and oxygen dependent enzyme system localized in the liver microsomes to yield metabolites tentatively identified as monomethyl norimipramine and hydroxy imipramine. Both of these metabolites however are further demethylated by the enzyme system to yield formaldehyde. These results are consistent with the following pathway:

imipramine ⟶ monomethyl norimipramine ⟶ norimipramine

↘

hydroxyimipramine ⟶ hydroxy monomethylnorimipramine

In view of these results, and the findings (see report by Sulser) that the monomethyl analogue is an extremely active drug, it is of obvious importance that an unknown metabolite of imipramine is present in the brain of rats given the drug 3 hours previously.

Significance to the Program of the Institute: These studies should provide a better understanding of the mechanism of action of imipramine.

Proposed Course of Project: The major metabolites formed by the microsomal enzyme system will be identified. We will determine whether imipramine is metabolized by tissues other than liver. In view of the lag in action of drug and the potency of the monomethyl analogue of imipramine (see Sulser report) it is important to establish whether the effects are mediated through this metabolite.

Part B included: No

Serial No. NHI-189
1. Chemical Pharmacology
2. Drug Enzyme Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Enzymatic Oxidation of Nicotine

Principal Investigators: Dr. Howard B. Hucker
Dr. James R. Gillette

Other Investigators: None

Cooperating Units: None

Man Years (Calendar Year 1960):
Total: 0.6
Professional: 0.6
Other: 0

Project Description:

Objectives: To study the enzymatic systems by which nicotine is metabolized in animals.

Methods Employed: We have developed differential solvent methods for the assay of nicotine, cotinine and γ(3'-pyridyl)-γ-methylaminobutyric acid (PMABA).

Major Findings: Nicotine is metabolized to cotinine by an enzyme system in rabbit liver microsomes that requires TPNH and oxygen. The reaction takes place in two steps: the first is the hydroxylation of nicotine to a cyclized aminoaldehyde (hydroxynicotine), and the second is the oxidization to cotinine. McKennis and co-workers had suggested that cotinine was formed by way of PMABA, but we found that no PMABA was formed during the oxidation of nicotine in a 9000 x g supernatant system. Moreover, we found that PMABA was formed in rabbits given cotinine or nicotine, but no cotinine was formed in rabbits given PMABA. Thus PMABA is formed by the hydrolysis of cotinine and not from the oxidation of γ (3'pyridyl)-γ-methylaminobutyraldehyde.

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Cotinine is quite stable in rabbits; it has a half life of about 6 hours. This could explain why we have found very little metabolism of cotinine with liver preparations.

Significance to the Program of the Institute: We have a better understanding of the metabolism of nicotine, a drug of considerable pharmacological importance.

Proposed Course of Research: This project has been terminated.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hucker, H. B., Gillette, J. R., and Brodie, B. B.: Enzymatic Pathway for the Formation of Cotinine, a Major Metabolite of Nicotine in Rabbit Liver. J. Pharmacol. Expt. Therap. 129, 94, 1960.

Serial No. NHI-190
1. Chemical Pharmacology
2. Drug Enzyme Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Mechanism of Binding of Drugs in Tissues

Principal Investigator: Dr. James R. Gillette

Other Investigators: Mr. James V. Dingell
Dr. William A. M. Duncan

Cooperating Units: Dr. Duncan was a guest workers from the Imperial Chemical Industries, Limited

Man Years (calendar year 1960):
Total: 0.8
Professional: 0.3
Other: 0.5

Project Description:

Objectives: A number of lipid soluble drugs are known to be bound by tissues. The purpose of this project is to discover what cellular components of the tissue bind the drugs, and to elucidate the mechanisms by which the binding takes place. These studies are important for several reasons: 1) Since most drugs are excreted and metabolized at a rate proportional to plasma level, the binding of drugs in tissues prolongs their duration of action by decreasing their free concentration in plasma and in cells in which they are metabolized. 2) The apparent activity of impure enzyme preparations is affected by the binding of the substrate to the tissue components. 3) The recovery of drugs and other substances can be affected by binding onto tissues.

Major Findings: About 80% of the imipramine added to plasma at therapeutic dosage is bound. The particulate fraction of 10% homogenates of brain or lung bind about 60-80% of the added imipramine or chlorpromazine. Moreover, 85% of imipramine added to 10% liver homogenates is bound to the particulate fractions; the specific activity (γ of drug per mg protein) was about ten times larger for the particulate matter than for the soluble fraction.

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Salzman and Brodie reported that only about 50% of the chlorpromazine added to brain homogenates could be recovered by their method. Unexpectedly, we found that nearly 90% of the chlorpromazine could be recovered if the heptane extract were shaken with 0.01 rather than the 0.1 M HCl suggested by the authors. Testing a number of substances known to be present in brain, we found that phosphatidyl inositol similarly prevented the return of chlorpromazine to 0.1 M HCl. Further study is necessary to explain this phenomenon.

Significance to the Program of the Institute: These studies should provide a better understanding of the factors affecting the duration of action of drugs.

Proposed Course of Project: We will study the effect of various concentrations of drugs to determine the binding capacity of the particulate components of liver. Attempts will be made to discover what chemical substances in the particulate fraction bind drugs.

Part B included: No

Serial No. NHI-191
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Chemical Inhibition of Cholesterol Biosynthesis

Principal Investigators: Dr. Elliott Schiffmann
Dr. Elwood O. Titus

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.77
Professional: 0.77
Other: 0

Project Description:

Objectives: A number of workers have determined the sequence of intermediates in the biosynthesis of cholesterol. This information has made it possible to design compounds which can inhibit the formation of cholesterol at specified points on the biosynthetic route. Interest has centered on analogs of mevalonic acid since the latter is readily and solely converted to cholesterol. It is probable that compounds which antagonize the formation of cholesterol from this important intermediate might not affect other metabolic processes.

The present study has the following objections: 1) To screen the inhibitory properties of a number of mevalonic acid analogs. 2) To compare the same compounds in vivo. 3) To study the metabolism of these compounds. 4) To determine their mechanism of inhibition.

Methods Employed: Various mevalonic acid analogs were prepared synthetically.

Isotopic cholesterol was isolated as the tomatinide which results in a more specific isolation of the sterol. The radioactivity of the product was determined by gas-flow and by scintillation counting procedures.

Major Findings: Three compounds suppress the incorporation of mevalonic acid-2-C^{14} into cholesterol in rat liver homogenates in the following order of potency: 3-hydroxy-3-methylvaleric acid Δ^2-3-methylpentenoic acid Δ^3-3-methylpentenoic acid.

In the ratio of 100 μmoles of the hydroxy acid to 1 μmole of substrate. The hydroxy acid produces 50% of the maximal inhibition. This compound appears to be a true inhibitor since it is not metabolized in liver homogenates. The specificity of the action of the hydroxy acid is indicated by studies on the metabolism of pyruvic acid; the formation of $C^{14}O_2$ from pyruvic acid-2-C^{14} in liver homogenates is not affected by a concentration of hydroxy acid which decreases the conversion of mevalonic acid-2-C^{14} into cholesterol by more than 60%.

$\Delta^{2,4}$-3-methyl pentadienoic acid has been synthesized and is being tested as an antimetabolite for mevalonic acid.

Significance to bio-medical research and the program of the Institute: Cholesterol metabolism might be involved in the development of atherosclerotic plaques. Supniewski et al. have reported that in both pigeons and rats the administration of Δ^3-3-methylpentenoic acid has resulted in the clearing of induced plaques from aortal tissue. A potent analog of mevalonic acid, such as the hydroxy acid, may have an advantage over the use of MER-29, which causes an accumulation of desmosterol.

Proposed Course of Project: 1) The preparation of fluorine containing analogs of mevalonate to be tested as inhibitors. 2) The enzymic sites of action of the effective analogs will be studied.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Honors and Awards: None

Publications:

Weiss, H., Schiffmann, E. and Titus, E. Effect of Analogs of Mevalonic Acid Upon the Biosynthesis of Cholesterol. J. Lipid Research, in press.

tissue fractions with possible radioactive precursors to trap any labeled product which might have been formed.

Major Findings: Following the incubation of slices, whole homogenate, and cytoplasmic fraction of rat liver with carbon-labeled glycine, formate, and adenine, no significant radioactivity was detectable in the N-methylated purines of the acid-soluble fraction.

In each of these tissue preparations, the label from methionine-CH_3-C^{14} was detectable in 6-MAP, and to a lesser extent in acid-soluble adenine, guanine and hypoxanthine. The total radioactivity of the methylated purine relative to the major purine bases was highest in the cytoplasmic system, and was enhanced both by drugs (azaserine, 6-thioguanine, 6-chloropurine) known to inhibit various steps in purine metabolism, and by the addition of ATP and Mg++ ion, which are required by the methionine-activating enzyme system.

Significance to bio-medical research and to the program of the Institute: The mechanism by which the information stored in desoxyribonucleic acid is transmitted to succeeding generations of cells is a basic problem in biochemistry. The presence of small amounts of methylated purines in DNA suggests: 1) That the resulting perturbation of the Watson and Crick structure of DNA may have gemetic significance. 2) That the methylated purine is an intermediate in the transfer of methyl groups of thymidine. This could explain the accumulation of methyl purine in the presence of thymidine antagonists and in certain mutants.

Proposed Course of Project: Experiments of the immediate future will be concerned with finding optimal conditions for the formation of 6-MAP.

Part B included: No

Serial No. NHI-193
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Metabolism of 6-Chloropurine

Principal Investigators: Dr. D. Duggan
Dr. E. Titus

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.8
Professional: 0.8
Other: 0

Project Description:

Objectives: The ultimate objective of this investigation is to elucidate the biochemical mechanisms by which 6-chloropurine (6-ClP), and possibly other related purine antimetabolites effect specific inhibitions of anabolic processes. To this end, studies of the metabolism of 6-ClP have been extended to isolated tissue systems and intracellular fractions, and investigations of the effects of 6-ClP and its metabolites on various synthetic systems in vivo and in vitro have been undertaken.

Methods Employed: The procedures already cited for measuring the effects of 6-ClP upon protein and lipid biosynthesis are being currently employed. Those relating to nucleic acid metabolism have been extended to provide for the isolation (paper chromatography) and quantitation of individual acid-soluble and nucleic acid purines, and for the precise determination of their radioactivities.

For studying the effects of 6-ClP and 6-chlorouric acid upon purine catabolizing systems, isotopic techniques based upon the recovery of the purine 6-carbon as $C^{14}O_2$ have been employed. In whole animal experiments, the conventional respiratory CO_2 procedure (Mac Kenzie, 1949) was used; for tissue slice and homogenate

systems, special micro-techniques were devised.

Major Findings: The limited utilization of 6-ClP for the biosynthesis of nucleic acid purines, and the nature of its urinary catabolites have been described in the previous report. Subsequent distribution studies have shown that the drug is cleared from the circulation with a half-time of about one hour, and accumulates to the greatest extent in liver. In contrast to other purine antimetabolites, a prolonged retention in tissues has been demonstrated.

The most pronounced metabolic effect of 6-ClP is upon the purine oxidizing enzyme systems. Therapeutic dosage of 6-ClP (80 mg/kg), or direct administration of its major metabolite, 6-chlorouric acid, markedly inhibited the xanthine oxidase - uricase pathway in the rat. In liver slices and homogenates, 50 per cent inhibition of xanthine oxidase, and almost quantitative inhibition of uricase are effected by concentrations of 6-ClP and 6-chlorouric acid corresponding to tissue levels in vivo.

6-ClP only slightly inhibits utilization of glycine-C^{14} in biosynthesis of liver protein and nucleic acid purines in vivo or in liver slices as measured by P^{32} incorporation. The compound inhibits RNA and DNA synthesis hardly at all. Total lipid synthesis in liver slices, however, as measured by acetate-1-C^{14} incorporation, is significantly inhibited by 6-ClP at levels of 100-200 μg/ml.

Significance to bio-medical research and the program of the Institute: Study of drug metabolism has been extended to include carcinostatic antimetabolites, which because of their similarity to normal purines, might be expected to act by virtue of their incorporation into more complex intermediates in nucleic acid synthesis. Metabolic studies may contribute to an understanding of the development of resistance to these drugs.

Proposed Course of Project: Experiments on the effects of 6-ClP on anabolic reactions in Ehrlich as cites in cells are in progress and will be completed.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Honors and Awards: None

Publications:

Duggan, D.E. and Titus, E. 6-Chloropurine and 6-Chlorouric Acid as Substrates and Inhibitors of Purine Oxidizing Enzymes. J. Biol. Chem. 234: 2100-2104 (1959).

Duggan, D.E. and Titus, E. The fate of 6-Chloropurine-8-C^{14} in the Rat. J. Pharmacol. and Exp. Therap. (in press, Dec. 1960).

Serial No. NHI-194
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Serotonin Transport in Platelets

Principal Investigators: Dr. Elliott Schiffmann
Dr. Elwood O. Titus

Other Investigators: None

Cooperating Units: Dr. Herbert Weissbach and staff
Laboratory of Clinical Biochemistry, NHI

Man Years (calendar year 1960)
Total: 0.43
Professional: 0.43
Other: 0

Project Description:

Objectives: In previous studies of the uptake of serotonin by blood platelets, Weissbach and Titus observed that active uptake occurred at pH 5.7. Both reserpine and cardiac glycosides inhibited this process. At higher pH (8.0), a diffusion process predominated, the latter being unaffected by the inhibitors.

As part of an investigation of the inhibitory mechanism a study of the binding of reserpine to platelets has been undertaken. This will include definition of conditions under which reserpine is bound as well as attempts to isolate cellular fractions to which the alkaloid is bound.

Methods Employed: Human platelets suspended in buffered saline were incubated with reserpine under different conditions. A sensitive method for reserpine assay allows accurate determination of binding.

Major Findings: Some observations were made upon the conditions under which reserpine is bound to platelets.

a) pH - The uptake of reserpine from a medium containing 50 γ/ml ranged from 20 to 40 γ of reserpine per mg of platelet protein. Variation of the pH from 5.7 to 7.4 does not change the amount of uptake of reserpine.

b) The uptake of reserpine was the same at 0° as at 38°.

c) Time of Incubation - The uptake of the alkaloid occurred within 5 minutes of mixing reserpine with the platelet suspension. The platelets could be centrifuged, washed in cold saline, and re-incubated in medium for 30 to 60 minutes without loss of bound reserpine.

d) Ouabain - Ouabain does not affect the uptake of reserpine by platelets.

Significance to bio-medical research and the program of the Institute: Active transport processes occur in platelets, a relatively simple and easily accessible system. Since cardiac glycosides and reserpine inhibit these active processes in vitro, the platelets provide a useful system for studies on mechanism of active transport.

Proposed Course of Project: 1) Some other parameters will be varied in order to determine their influence upon reserpine uptake. 2) Attempts will be made to determine whether reserpine is bound to a non-diffusible component of platelets. 3) It is hoped to be able to isolate and characterize cellular fractions to which reserpine is bound.

Part B included: No

Serial No. NHI-195
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on an Aldosterone Stimulating Hormone

Principal Investigators: Dr. Elwood O. Titus
Dr. James O. Davis

Other Investigator: Mr. Herbert E. Spiegel

Cooperating Units: Laboratory of Kidney and Electrolyte Physiology, NHI

Man Years (calendar year 1960)
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:

Objectives: There is evidence that one or more humoral mediators, in addition to ACTH, stimulate secretion of aldosterone by the adrenal cortex in the secondary hyperaldosteronism of congestive heart failure, after periods of low sodium intake, or after constriction of the inferior vena cava.

Experiments carried out in the Laboratory of Kidney and Electrolyte Physiology indicate that the kidney produces a stimulatory factor in response to caval constriction. Isolation of this factor is being undertaken.

Methods Employed: The rate of secretion of aldosterone in the adrenal venous blood of hypophysectomized dogs is measured by an isotope derivative method before and after injection of kidney extracts.

Major Findings: Saline extracts of dog kidney stimulate secretion of aldosterone. The active material is stable to heat at pH 6.4. After heat treatment at 55° the extracts stimulate secretion of corticosterone, presumably a precurser of aldosterone, as well as of aldosterone. Fractionation by alcohol precipitation

and by dialysis indicate the presence of two active factors one of which is dialyzable.

At least some of the activity is probably due to renin, since hypertensin, the product that appears in circulating blood after liberation of renin, stimulates aldosterone secretion. Partially purified renin stimulates aldosterone secretion. Renal hypertensive dogs, however, do not exhibit increased aldosterone secretion. The relationship between the renin-hypertensin system and the aldosterone stimulating mechanism is not yet clear.

Significance to bio-medical research and to the program of the Institute: Secondary aldosteronism of congestive heart failure with its sequelae of sodium retention and edema is a serious complication. An understanding of its mechanism is of both practical and theoretical interest.

Proposed Course of Project: 1) Efforts to isolate the kidney factor will continue. 2) Immunochemical methods will be used to determine whether the renin-hypertensin system is involved in the biosynthesis of aldosterone.

Part B included: No

Serial No. NHI-196
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Mechanism of Uptake of Catecholamine by Brain and Sympathetically Innervated Tissues

Principal Investigators: Dr. Elwood O. Titus
Dr. Hans J. Dengler (NIH Postdoctoral Fellow, from University of Heidelberg, Heidelberg, Germany).

Other Investigator: Mr. Herbert E. Spiegel

Cooperating Units: None

Man Years (calendar year 1960)
Total: 2.25
Professional: 1.5
Other: 0.75

Project Description:

Objectives: It is important to understand mechanism by which relatively high concentrations of catecholamines are maintained in cells since many psychotropic and hypotensive agents change the tissue levels of catecholamine. Of particular interest is the possibility that active transport contributes to the maintenance of these levels. Present studies include:

1) Kinetics of norepinephrine uptake in tissues exposed to the labelled amine. This should provide evidence concerning the intracellular pools of norepinephrine and the manner in which the amine is distributed among them.

2) Effects of various drugs on the uptake mechanism. Appropriately chosen drugs should reveal not only the effect of the drug on uptake, but also the role that the uptake mechanism performs in the function of adrenergic organs.

Methods Employed: Chromatographic and extraction procedures are used to determine the amounts and specific activities of

tritium labelled catecholamines and their metabolites.

Major Findings: Kinetic data indicate that norepinephrine enters cells of cat cerebral cortex, heart, and kidney both by diffusion and by a concentrating mechanism. The latter resembles active transport in that it can be inhibited by cardiac glycosides and approaches a limiting rate as it becomes saturated at increasing norepinephrine levels. Uptake by this mechanism is maximal at approximately 10-25 mμg/ml.

The concentrating mechanism is absent in liver, muscle or adrenal slices, but is very active in the pineal body. Tissues from animals that have received reserpine or morphine, but not from those receiving amine oxidase inhibitors do not concentrate norepinephrine. Thyroxine given in vivo inhibits uptake by the heart.

A number of drugs inhibit uptake in vitro. These include both sympathomimetic and sympatholytic agents structurally analogous to norepinephrine; a number of other sympatholytic agents, but not dibenamine; depleters of tissue catecholamines such as reserpine and guanethidine; cocaine, a sympathomimetic agent; bretylium, an agent which interferes with physiological release of norepinephrine, and tofranil, an antidepressant.

Almost all of the inhibitory compounds are known to enhance the response of adrenergic organs to catecholamines. It is possible that the uptake mechanism is a means for terminating the biological action of norepinephrine and epinephrine. It appears to be specific for the latter compounds; isotopic dopamine is not taken up by the tissues at comparable concentrations.

Chemical studies show that the isotope taken into cortex and heart remains there almost entirely as unchanged norepinephrine but is discharged from the cortex entirely as the products of amine oxidase action and from the heart entirely as normetanephrine.

Significance to bio-medical research and to the program of the Institute: An understanding of the molecular events at the receptor site including the means by which catecholamines are stored and released to reach their target organs is prerequisite to an understanding of the mechanism of action of these substances as well as of many drugs.

Proposed Course of Project: Studies of the kinetics of norepinephrine uptake into various subcellular fractions from brain and other tissues will be continued. Work will be designed

to establish:

1) Whether reserpine and other depletors of catecholamine act at the cell membrane or directly at the storage sites in intracellular particles.

2) Whether other drugs which influence the function of sympathetically innervated organs change the amounts or intracellular distribution of tissue catecholamines.

Part B included: No

Serial No. NHI-197
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Use of Cardiac Glycosides in Studies of Membrane Function

Principal Investigators: Dr. Doris Nicholls (Guest worker from the University of Western Ontario, Ontario, Canada)
Dr. Elwood O. Titus

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1.0
Professional: 1.0
Other: 0.0

Project Description:

Objectives: The cardiac glycosides are useful in studying the mechanism of cation transport through membranes since there is abundant evidence that these steroids inhibit the utilization of metabolic energy for cation transport without affecting the synthesis of ATP.

Recently phosphatidic acid, a phospholipid which occurs in minute amounts in most cells and which turns over extremely rapidly, has been postulated to serve as a carrier of sodium ions through cell membranes. Should this be so, it might be expected that the cardiac glycosides would slow the turnover of this phospholipid. The effects of ouabain on the incorporation of labeled phosphate into phosphatidic acid are being studied in several tissues.

Methods Employed: Conventional methods for isolation of phospholipid and counting of radioactivity. Paper chromatographic identification of phosphatidic acid.

Major Findings: The synthesis of labeled phosphatidic acid from exogenous phosphate is relatively slow in slices of rabbit heart and diaphragm, faster in liver and kidney, and most rapid in brain. The rate of synthesis is appreciable in slices from the salt gland of the sea gull. The effects of steroids have been studied in most detail in rabbit brain slices, but the following conclusions apply to all tissues examined. Ouabain inhibits the synthesis of phosphatidic acid at concentrations as low as 10^{-7} M. The extent of inhibition approaches 50 per cent between 10^{-6} and 10^{-5} M ouabain and is not further increased at ouabain levels up to 10^{-4} M.

There is a lag period before the rate of incorporation of labeled phosphate into phospholipid becomes maximal. This lag may be due to the low permeability of the cell to inorganic phosphate. High levels (10^{-5} M) of ouabain eliminate this lag, apparently by enhancing permeability to phosphate in a manner similar to the action of certain detergents, freezing and thawing, or addition of acetyl choline.

Significance to bio-medical research and to the program of the Institute: The controlled transport of cations through membranes plays a vital role in the functions of the cardiovascular and nervous systems. Although it is clear that a number of steroid hormones act by regulation of cation transport essentially nothing is known of the biochemical mechanisms involved.

Proposed Course of Project: A previous report has established that certain red cells with cation transport systems that are inhibited by cardiac glycosides do not synthesize phosphatidic acid. This suggests that the turnover of phosphatidic acid may not be a necessary part of cation pumping. The sensitivity of the phenomenon to steroids and to the monovalent cations in the media, however, suggest that in tissues more complicated than red cells the synthesis of phosphatidic acid is in some way dependent on the functioning of the transport system. This possibility will be investigated.

It is noteworthy that inhibition of the phosphatidic acid synthesis never exceeds 50 per cent. Recently a number of tissues have been found to contain phosphatides chromatographically very similar to phosphatidic acid. The possibility that the steroid is inhibiting the synthesis of only certain components of a more complex mixture will be investigated.

Part B included: No

Serial No. NHI-198

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Incorporation of palmitate-1-C^{14} into triglycerides and phospholipids by cell-free homogenates of livers from control and ethanol-treated rats.

Principal Investigators: Dr. Masao Wakabayashi
Dr. Harriet Maling

Other Investigators: Dr. Marjorie Horning, LCPM, NHI

Cooperating Units: LCPM, NHI
Dr. Wakabayashi is a Seagram Fellow

Man Years (calendar Year 1960):
Total: 0.7
Professional: 0.7
Other: None

Project Description:

Objectives: To study the incorporation of albumin-bound palmitate-1-C^{14} into triglycerides and phospholipids by cell-free homogenates of livers from control rats and rats which have been pretreated with ethanol. These experiments may give insight into the mechanisms responsible for triglyceride deposition in liver after ethanol.

Methods Employed: To study triglyceride synthesis in vitro albumin-bound palmitate-1-C^{14} is incubated with appropriate cofactors with cell-free homogenates of livers from control fed and fasting rats and from fasting rats killed 7 hours after the oral administration of ethanol (6 g/kg). The lipids are extracted from the incubation mixture. The radioactivity of total lipids and the lipid fractions is determined.

Major Findings: Liver homogenates from ethanol-treated rats show an enhanced incorporation of palmitate-1-C^{14} into triglycerides and a decreased incorporation into phospholipids. These results suggest that stimulation of pituitary-adrenal system by alcohol with resultant fatty liver might also involve activation of enzymes that synthesize neutral fat.

Proposed Course of Project: A study will be made of the incorporation of palmitate-1-C^{14} by cell-free homogenates of livers from ethanol-treated hypophysectomized rats, ethanol-treated intact rats, and ethanol-treated rats which have been pretreated with chlorisondamine, a ganglionic blocking agent which effectively prevents ethanol-induced triglyceride deposition in rat liver. Similar experiments will be done in vivo.

Part B included: No

Serial No. NHI-199
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Digitalization Upon the Minimal Effective Dose of Barbiturate Required to Produce a Negative Inotropic Effect in Dogs

Principal Investigator: Dr. Harriet Maling

Other Investigator: Mrs. Martha A. Williams

Cooperating Units: Dr. Alan H. Goldberg, Anesthesiology Dept., Clinical Center
Dr. Thomas Gaffney, Section on Cardiology, Surgery Branch, NHI

Man Years (calendar year 1960):
Total: 0.1
Professional: 0.05
Other: 0.05

Project Description:

Objectives: To determine whether digitalization influences the minimal dose of barbiturate required to cause a negative inotropic effect.

Preoperative administration of cardiac glycosides to patients without evidence of congestive heart failure is based upon the reasonable conjecture that the positive inotropic effect of cardiac glycosides would benefit the heart "stressed" by anesthesia and surgery. It is known that barbiturates have a negative inotropic effect upon the heart. As part of the general question of the value of preoperative digitalization in patients without overt evidence of congestive heart failure, it is important to know if digitalization alters quantitatively or qualitatively the response of an animal to thiopental and other common anesthetic agents.

Methods Employed: A strain gauge arch is placed on the heart of a dog which has been anesthetized with chloralose. Thiopental sodium is infused intravenously. Myocardial contractile force, arterial blood pressure, venous pressure, and electrocardiograms are recorded before and during the infusion. A comparison is made of the results of this procedure in control and digitalized dogs.

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Major Findings: Digitalization with small doses (0.025 mg/kg I.V.) of digoxin did not modify significantly the response to the thiopental infusion. Digitalization with large doses (0.2 mg/kg I.V.) delayed the fall in contractile force, but also produced ECG signs of toxicity (scattered ectopic beats). The effects of intermediate doses of digoxin are now under study.

Significance to the Program of the Institute: This study should interest those persons who advocate preoperative digitalization of patients.

Proposed Course of Project: The outlined experiments will be completed.

Part B included: No

Serial No. NHI-200
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Fat Mobilization and Triglyceride Deposition in Rat Liver after Diverse Agents

Principal Investogator: Dr. Harriet Maling
(Dr. Roger Maickel -- see separate report)

Other Investigators: Mr. William M. Butler, Jr.
Mrs. Martha A. Williams
Mrs. Jeannette Hunter

Cooperating Units: Dr. Marjorie Horning, LCPM, NHI
Dr. Benjamin Highman, NIAMD

Man Years (calendar year 1960):
Total: 2.0
Professional: 0.7
Other: 1.3

Project Description:

Objectives: To study mechanisms involved in drug-induced mobilization and deposition of neutral fat. To determine the role of pituitary and autonomic nervous system in mobilization and deposition of triglycerides. To study the effects of autonomic drugs upon fat mobilization and deposition.

Methods Employed: The triglyceride content of rat liver is measured directly by our modification of the method of van Handel and Zilversmit. Plasma free fatty acids and plasma triglycerides are also measured.

Major Findings: Triglyceride levels in rat liver are considerably elevated within 24 hours after administration of catecholamines, cortisone, ethanol, ethionine and carbon tetrachloride. Maximum triglyceride levels measured after various combinations of catecholamines and cortisone are considerably less than after the other agents. Triglyceride deposition is consistently greater in female rats than in male rats; the sex difference is most marked with ethionine, but definite also with carbon tetrachloride and ethanol. Deposition has been histologically confirmed and extended to other tissues.

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Measurements by gas chromatography of the fatty acid composition of liver triglycerides after the administration of ethanol, carbon tetrachloride, or ethionine, show that the concentrations of linoleic and oleic acids in the deposited fat are about the same as in adipose tissue. Since rat liver cannot synthesize linoleic acid, this increase in triglyceride linoleic acid indicates mobilization of fatty acids from adipose tissue.

Hypophysectomy completely prevents triglyceride deposition after ethanol and partially the deposition induced by ethionine, and carbon tetrachloride. In intact rats, administration of ethanol causes a pituitary response, as indicated by a depletion of adrenal gland ascorbic acid, elevations in plasma corticosterone and free fatty acids, and an increased liver tryptopyan peroxidase (see report of Dr. Roger Maickel).

Pretreatment with Dibenamine reduces slightly the triglyceride deposition after ethanol and markedly the deposition after ethionine and carbon tetrachloride. Pretreatment with the ganglionic blocking agent, chlorisondamine, reduces markedly the triglyceride deposition after ethanol, but is less effective in preventing the deposition after ethionine or carbon tetrachloride.

Significance to the Program of the Institute: This project increases our understanding of the mechanisms involved in triglyceride mobilization and deposition and how drugs cause a fatty liver. Triglyceride deposition may be involved in development of chronic diseases, such as hepatic cirrhosis and perhaps atherosclerosis.

Proposed Course of Project: The effects of various treatments upon triglyceride levels in rat liver will be correlated with changes in plasma free fatty acids and plasma triglycerides. Triglyceride deposition will be studied in hypophysectomized rats, demedullated rats, and in rats with other ablations.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Maling, H. M., Highman, B. and Thompson, E. C. Some similar effects after large doses of catecholamines and myocardial infarction in dogs. American Journal of Cardiology, 5: 628-633, 1960.

Butler, W. M., Jr., H. M. Maling, M.G. Horning and B. B. Brodie. The direct determination of liver triglycerides. J. Lipid Research, in press.

Brodie, B. B., W. M. Butler, Jr., M. G. Horning, R. P. Maickel and H. M. Maling. Alcohol-induced triglyceride deposition in liver through derangement of fat transport. Am. J. Clin. Nutrition, in press.

Brodie, B. B., H. M. Maling, M. G. Horning and R. P. Maickel. Triglyceride deposition in rat liver through derangement of fat transport by alcohol and other chemical agents. Proc. Internat. Sym. on Drugs Affecting Lipid Metabolism, Milan, Italy, June 2-4, 1960, in press.

Horning, M. G., Williams, E. A., Maling, H. M., and Brodie, B. B. Depot fat as source of increased liver triglycerides after ethanol. Biochem. and Biophys. Res. Comm., in press.

Serial No. NHI-201
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The effect of CCl_4-poisoning upon hepatic release of labeled triglycerides after intravenous injection of palmitate-1-C^{14} in rats

Principal Investigators: Mr. Arthur Frank
Dr. Harriet Maling

Other Investigators: Dr. Marjorie Horning, LCPM, NHI

Cooperating Unit: LCPM, NHI

Man Years (calendar year 1960):
Total: 0.3
Professional: 0.05
Other: 0.25

Project Description:

Objectives: To investigate the possibility that blocking of hepatic release of triglyceride might contribute to the elevated liver triglyceride levels after carbon tetrachloride.

Methods Employed: Albumin-bound palmitate-1-C^{14} is injected into the tail vein of control fed and fasting rats and in fasting rats about 20 hours after the oral administration of carbon tetrachloride. The radioactivity of total plasma lipids and of plasma lipid fractions is determined 10, 20, 30, 40 and 60 minutes after injection of the labeled palmitate.

Major Findings: Within 10 minutes almost all radioactive palmitate disappears from plasma of control and CCl_4-treated rats; this indicates that the livers and other tissues of treated rats are removing circulating fatty acids normally. In control rats, most of the radioactivity of plasma appears in the triglycerides. Peak values of radioactivity in plasma triglycerides occur 20 to 30 minutes after injection of labeled palmitate in control fed and fasting rats. No peaks can be detected in CCl_4-treated rats, and there is almost no radioactivity in the plasma triglycerides. The results are most easily interpreted in terms of a marked block of hepatic release of triglycerides. This block may be largely responsible for the accumulation of triglycerides in the livers of CCl_4-treated rats. This was

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verified by the observation that liver triglycerides of normal rats contained much less radioactivity than those of CCl_4-treated animals.

Proposed Course of Project: The radioactivity of liver and adipose tissue will be measured to check the assumption that the liver rapidly removes the bulk of the injected labeled palmitate. The radioactivity of the liver triglycerides will be measured to check that the livers of CCl_4-treated rats can form triglycerides from fatty acids as well as the control rats. We shall also study the effects of hypophysectomy and pretreatment with Dibenamine upon hepatic release of triglycerides in CCl_4-treated rats.

Part B included: No

Serial No. NHI-202
1. Chemical Pharmacology
2. Section on Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Pharmacology of JB 516 and Other Inhibitors of Monoamine Oxidase.

Principal Investigators: Dr. Harriet M. Maling
Dr. Sydney Spector

Other Investigators: Mrs. Martha A. Williams
Mr. Duffy E. Mc Brayer

Cooperating Unit: Dr. Benjamin Highman, NIAMD

Man Years (Calendar Year 1960):
Total: 1.0
Professional: 0.4
Other: 0.6

Project Description:

Objectives: To correlate changes in behavior and neurologic symptoms (see Annual Reports for 1958 and 1959) with pathologic findings and levels of serotonin and norepinephrine in various parts of CNS.

Methods Employed: Described in annual reports for 1958 and 1959.

Major Findings: Prolonged parenteral administration of JB 516 to dogs, cats, and squirrel monkeys consistently produced marked neurologic symptoms, including ataxia and impaired motor coordination. Other neurologic symptoms varied with species, dose, and total dosage. In dogs, other symptoms will include hyperesthesia, priapism, stiffness of legs, extensor spasms of front legs, sinking of hind legs, unsteadiness during standing, tremors at rest, uncontrolled jerking of head, hoarse barking, nystagmus, and lowered temperature. Marked neurologic symptoms also developed consistantly in dogs after prolonged parenteral administration of JB 835. With other inhibitors, marked neurologic symptoms did not develop consistently, but were observed in one or more dogs with each of the following: iproniazid, phenelzine, desoxyephedrine hydrazide, Marplan, Nialamid, and MO 911. Most dogs developed some milder symptoms, such as stiffness in the hind legs and hyperesthesia.

Subcutaneous administration of doses of MO 911 greater than 20 mg/kg could not be continued in dogs for long periods because of marked tissue necrosis around injection sites. Daily parenteral administration of large

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doses of Marplan and Nialamid was difficult because of the poor solubility of these compounds.

Prolonged oral administration of large doses of MO 911, Nialamid and Marplan was well tolerated by most dogs. However, marked neurologic symptoms developed in 1 dog after 4 oral doses of 75 mg/kg Nialamid, and in another dog which received 32 oral doses of 20 mg/kg Marplan. Marked neurologic symptoms developed in all 4 dogs given large oral doses (4 mg/kg) of JB 516 daily for long periods.

After prolonged administration of JB 516 or JB 835, most dogs developed neurologic lesions. The most constant lesions were in the inferior olivary nucleus and in the pyriform lobe. In the inferior olivary nucleus, many ganglion cells showed large cytoplasmic vacuoles, some were shrunken, and some had pyknotic nuclei. In the pyriform lobe, many neurones showed chromatolysis and a diffuse eosinophilic staining. Such marked neuropathologic changes have not been observed in cats, rabbits, and squirrel monkeys given JB 516, or in dogs given a number of other monoamine oxidase inhibitors, including MO 911, iproniazid, Nialamid, and Marplan; however, neuropathologic studies with these other inhibitors are still incomplete. Pyridoxine did not prevent the neurologic symptoms or lesions in dogs given JB 516.

Prolonged administration of large doses of JB 516, JB 835, MO 911, phenelzine, Nialamid and Marplan produced comparably high elevations of serotonin in the brain-stems and spinal cords of dogs; norepinephrine levels were not significantly changed. JB 516 elevated both the norepinephrine and serotonin levels in brain-stems and spinal cords of rabbits and squirrel monkeys. In cats and dogs, only the serotonin levels were increased.

Significance to the Program of the Institute: Neurologic symptoms and neuropathologic lesions produced by monoamine oxidase inhibitors are of great interest because some of these inhibitors are being tried clinically in the treatment of hypertension and angina pectoris.

Proposed Course of Project: Manuscripts are now in preparation.

Part B included: No

Serial No. NHI-203
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Relationship Between Reserpine Action and Limbic System Excitability

Principal Investigators: Dr. E. Costa
Dr. A. Revzin
Dr. S. Spector

Other Investigator: None

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: .1
Professional: .1
Other: None

Project Description:

Objectives: The importance of biogenic amines in regulating limbic excitability is studied with regard to: (a) the responses of limbic system to peripheral stimuli (b) the responses of limbic structures to activation by other limbic components, and (c) the characteristics of outflow of nerve impulses from limbic structures.

Methods Employed: Standard neurophysiological evoked and unit potential techniques are applied to rats, rabbits and cats given reserpine or other drugs which mimic selected pharmacological and biochemical effects of this alkaloid.

Major Findings: Reserpine greatly increases the excitability of cat hippocampus by electrical stimulation of amygdala. In consequence, this drug strikingly reduces the convulsive thresholds of this structure. Amphetamine reduces the increased excitability of the hippocampus induced by reserpine but in normal cats does not by itself alter the excitability of this structure.

Significance to the Program of the Institute: The "Limbic System" is the highest center of integration, or the head ganglion, of the autonomic nervous system, as well as a major CNS substrate for the expression of emotion and selection of appropriate behavior to fit in the continuous variability of the environment. It is not surprising that reserpine which causes neuroleptic

effects and alters the reflectivity of the CNS representation of the autonomic system, induces effects on these structures. This study will add to the understanding of the importance that changes of amines in the limbic system have in the mode of action of drugs related to reserpine used in the therapy of cardiovascular and neurological diseases.

Proposed Course of Project: 1) Study of the effects of pharmacological analogs of reserpine on limbic system; 2) Study of the changes of limbic excitability caused by α-methyl-meta-tyrosine that selectively reduces brain norepinephrine content; 3) Study of the action of MAO inhibitors in normal animals and animals pretreated with α-methyl-meta-tyrosine, and/or given reserpine, to investigate the actions of free serotonin.

Part B included: No

Serial No. NHI-204
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Mechanisms of Action of Guanethidine

Principal Investigators: Dr. E. Costa
Dr. L. Gessa
Mr. R. Kuntzman

Other Investigator: None

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: .2
Professional: .2
Other: None

Project Description:

Objectives: Guanethidine has been shown to be useful in the treatment of hypertension; but the mechanism through which Guanethidine produces this decrease in blood pressure is not known. This study is designed to elucidate this mechanism.

Methods Employed: Standard pharmacologic and chemical procedures.

Major Findings: Guanethidine has been found to deplete peripheral norepinephrine. In this way chemical sympathectomy is produced, with a consequent effect on blood pressure. Unlike reserpine, which also depletes norepinephrine, Guanethidine has been found not to decrease serotonin levels in the body and not to decrease the norepinephrine content of the brain. Since Guanethidine has a very low lipid solubility, the drug does not pass through the blood-brain barrier. This accounts for the absence of any effects on centrally stored norepinephrine.

Significance to the Program of the Institute: Our results indicate that Guanethidine can be used as a tool to study differential mechanisms in storage and release of selected biogenic amines in cardiovascular system. Since the release of amines by nerve impulses is a factor which modulates tonus of peripheral autonomic system, this study may help to understand control of cardiovascular function by autonomic nervous system.

Proposed Course of Project: The production of chemical sympathectomy by Guanethidine has been shown. Therefore, it is of interest to study how the depletion of peripheral norepinephrine is accomplished, since this mechanism seems different from that of reserpine, which has been shown to impair the storage of both norepinephrine and serotonin.

Part B included: Yes

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Cass, R., Kuntzman, R., and Brodie, B.B.: Norepinephrine Depletion as a Possible Mechanism of Action of Guanethidine (SU 5864), a New Hypotensive Agent. Proc. of Soc. for Expt. Biol. and Med. 103: 871-872, 1960.

Brodie, B.B., and Kuntzman, R.: Pharmacological Consequences of Selective Depletion of Catecholamines by Antihypertensive Agents. Ann. N. Y. Acad. Sci. 88: 939-943, Oct. 11, 1960.

Serial No. NHI-205
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Variation in Norepinephrine and Serotonin Levels and the Enzymes Responsible for Their Synthesis and Metabolism in Fetal and Immature Animals.

Principal Investigator: Dr. N. Karki

Other Investigator: Mr. R. Kuntzman

Cooperating Unit: Dr. Karki had Fellowship from Finland.

Man Years (Calendar Year 1960):
Total: .7
Professional: .7
Other: None

Project Description:

Objectives: Since the concentration of norepinephrine and serotonin in the brains of newborn rats is only a small fraction of the concentration of these amines found in adult, it is of interest to determine if the lower levels of these amines are related to enzyme concentrations. It is also of interest to study these same parameters in other species, from birth to adulthood.

Methods Employed: Standard pharmacologic and chemical procedures.

Major Findings: The brain levels of NE and 5HT in newborn guinea pigs, which unlike newborn rats, have well-defined behavioral patterns, the same as in the adult. Some of the enzymes responsible for synthesis and metabolism of these amines were studied in guinea pigs and rats. The activity of DOPA-5HTP decarboxylase was the same in newborn rats and guinea pigs as in the adults of these species. Monoamine oxidase activity in newborn rats was only 25% of that found in the adult; however, this finding is in the wrong direction to explain the lower amine levels. In contrast, the MAO activity was the same in newborn and adult guinea pigs. Since enzymatic activity did not account for the lower amine levels in newborn rats, the storage capacity of the brains for these animals was studied. The administration of a MAO inhibitor to newborn and adult rats and guinea pigs indicated that whereas the newborn guinea pig could store as much of these amines as the adult, newborn rats

could store only a small fraction of the amount stored in the adult. Therefore, a deficiency in the ability to store norepinephrine and serotonin seems responsible for the lower amine levels found in the brains of newborn rats.

Significance to the Program of the Institute: Since norepinephrine and serotonin are postulated to be neurohormones it is important to study the relationships of these substances to one another in developing animals.

Proposed Course of Project: Since norepinephrine and serotonin are found only in small amounts in developing rats, it is of interest to investigate in these animals the effect of drugs that act on the brain through changes of curine content. In addition, norepinephrine and serotonin will be studied in various species to gain more information concerning their role as neurohormones.

Part B included: No

Serial No. NHI-206
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Role of Monoamine Oxidase in the Ganglion

Principal Investigators: Dr. E. Costa
Dr. S. Spector
Mr. R. Kuntzman
Dr. A. Revzin

Other Investigator: Mrs. Carolynne Hirsch

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: .4
Professional: .3
Other: .1

Project Description:

Objectives: The present study is designed to evaluate the relationship between MAO content of ganglia and transmission of impulses through ganglionic synapses.

Methods Employed: Standard neurophysiological techniques to evaluate ganglionic transmission, a micromethod to determine catecholamines in ganglia, and a modification of the *in vitro* assay for MAO in order to assay the enzyme activity in the ganglia.

Major Findings: In rabbits a monoamine oxidase inhibitor (MO 911) produces in 18 hours a 60% increase in norepinephrine content of ganglia. Larger increases were obtained by chronic administration.

In cats the content of the ganglia did not increase even after chronic administration of MO 911. In both species there was a short-lasting blockade of transmission. However this effect had disappeared when MAO inhibitor and rise in amines were maximal. These results indicate that MAO inhibitors do not lower blood pressure by their affects at ganglia.

Significance to the Program of the Institute: These studies may add to our knowledge of the mechanism of action of MAOI lower blood pressure.

Proposed Course of Project: To determine the effects of a pretreatment with MAOI on the ganglionic action of nicotine and other drugs which release catecholamines in ganglion.

Part B included: No

Serial No. NHI-207
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Pharmacological Analysis of the Action of Reserpine and Chlorpromazine on the Autonomic Nervous System

Principal Investigator: Dr. F. Sulser

Other Investigator: Mr. James Watts

Cooperating Unit: Dr. F. Sulser is a NIH Post-doctoral Research Fellow.

Man Years (Calendar Year 1960):
Total: 0.9
Professional: 0.4
Other: 0.5

Project Description:

Objectives: Further studies on reserpine induced parasympathetic syndrome.

Major Findings:
1) Effects on autonomic nervous system

Previously we reported that reserpine evokes increased parasympathetic activity in absence of sympathetic innervation but requires an intact parasympathetic nerve supply. Thus miosis, lacrimation and salivation are due to an increased parasympathetic output and not due to a decreased sympathetic output as hitherto considered. This suggests that reserpine and chlorpromazine act on different neural systems.

Additional evidence of this was from studies showing that chlorpromazine does not evoke lacrimation or salivation. Thus central adrenergic blockade by chlorpromazine does not result in an increased parasympathetic output.

Pharmacological independence of the central sympathetic and parasympathetic systems is also shown by failure of amphetamine, a centrally acting sympathomimetic agent, to decrease the reserpine-induced flow of saliva.

Additional evidence that reserpine evokes a generalized stimulation of central parasympathetic system is suggested by the antagonism of Ecolid for reserpine-induced diarrhea in rabbits and rats, despite almost complete release

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of gut 5HT.

2) Stimulatory effects of reserpine on neural system

Indirect evidence indicates that reserpine exerts its action by a stimulatory effect on the CNS. Thus reserpine elicits almost no salivation in dogs anesthetized with pentobarbital or chloralose. Moreover reserpine-induced salivation is abruptly slapped when the anesthetics are given. Even the enhancement of light reflex induced by reserpine is blocked by anesthesia.

3) Short-lived parasympathetic effects

For reasons, still unclear, the salivation and direct miosis (miosis in dark room) produced by reserpine terminate in a few hours when animals are most deeply sedated, but enhancement of light reflex persists. A second dose of reserpine does not reactivate flow of saliva. Reasons for the apparent dissociation of parasympathetic effects from the tranquilization is important in understanding interaction of reserpine with 5HT. The working hypothesis is advanced that sedation produced by reserpine acts like anesthesia to block its own effects. When the animals were also given amphetamine salivation does not terminate in 5 hours but continued as long as animals were kept aroused. The possibility was considered that salivation stopped when 5HT was depleted. But imipramine blocks salivation without preventing release of 5HT. Yet when effects of imipramine wear off, salivation starts despite absence of 5HT store.

4) Are parasympathetic effects mediated through 5HT?

a) 5HTP elicits salivary flow which is prevented by a ganglionic blocker (Ecolid) and by preganglionic section of chorda tympani.

b) α-methyl dopa, a decarboxylase inhibitor, both prevents and stops reserpine-induced salivation, without affecting response to peripheral cholinergic stimuli. In contrast to Ecolid, the amino acid had no effect on parasympathetic ganglia.

5) Does reserpine act at parasympathetic ganglia?

The possibility that part of reserpine action is due to enhancement of ganglionic synaptic transmission was studied by effect of reserpine on postganglionic response of effector organ to preganglionic stimulation. Results are inconclusive because of variability of controls.

6) Action of Tetrabenazine

This benzoquinolizine elicits reserpine-like effects. However pharmacological effects occur quickly; salivation is maximal within 1 hour compared to 3 hours for reserpine. Furthermore the flow of saliva is much more copious after tetrabenazine.

The finding that tetrabenazine acts rapidly yet releases brain amines at same rate as reserpine; suggests a different mode of action for former drug. A number of lines of evidence suggest that tetrabenazine has a mixed action through mimicking 5HT and releasing 5HT:

a) Tetrabenazine produces profound sedation and other reserpine-like effects at doses that release less than 50% of brain 5HT. Reserpine analogs

produce central effects only with a 5HT lowering in brain of about 55%.

b) When salivary response ceases after tetrabenazine, another dose restarts the flow. Even after reserpine administration, tetrabenazine restarts the flow.

Significance to the Program of the Institute: This project is designed to elucidate the action of reserpine on the central autonomic nervous system and may help to understand the role of certain important amines in autonomic function under physiological and pharmacological conditions.

Proposed Course of Project:

1) To study the role of 5HT in the reserpine-induced increase of parasympathetic activity.

2) To measure "traffic" in preganglionic parasympathetic nerves e.g. chorda tympani after reserpine and in preganglionic sympathetic fibres after chlorpromazine.

3) To study the mechanism of endogenous HT on peristalsis and the relationship between reserpine induced diarrhea and release of intestinal HT.

4) To study the role of cholinergic or "Serotoninergic" pathways for the release of ACTH after the administration of reserpine with and without imipramine as a hypothetical blocker of centrally mediated increase of parasympathetic activity.

Part B included: Yes

Serial No. NHI-207

1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Bogdanski, D. F., Sulser, F., and Brodie, B. B.: A Pharmacological Analysis of the Action of Reserpine on the Central Autonomic Nervous System. J.P.E.T., in press.

Serial No. NHI-208
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Function of Norepinephrine in Sympathetic Synaptic Transmission

Principal Investigators: Dr. E. Costa
Mr. R. Kuntzman
Dr. A. Revzin
Dr. S. Spector

Other Investigators: Mrs. Carolynne Hirsch

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: 1.2
Professional: 1.0
Other: 0.2

Project Description:

Objectives: The present study was designed to study the role of NE in ganglionic sympathetic transmission. The depletion of catecholamine by reserpine or the increase of free catecholamine by MAOI + reserpine was studied on the transmission of impulses through the superior sympathetic cervical ganglion of cats and rabbits.

Major Findings: Reserpine in cats and rabbits decreases ganglionic norepinephrine content. In both species a decrease of the catecholamine content greater than 90% is elicited by doses of reserpine greater than .6 mg/kg i.v. The postganglionic evoked potential is enhanced within 4 hours by doses of reserpine greater than .6 mg/kg i.v. Maximal effects are obtained with 1.25 mg/kg of the alkaloid and the effects last 20 hours or more. Bretylium which prevents physiological release of norepinephrine at nerve endings, and two adrenergic blocking agents, ergotamine and dibenamine, also facilitate postganglionic evoked potentials. If ganglionic norepinephrine is liberated by reserpine after pretreatment with a MAO inhibitor, ganglionic transmission is almost completely blocked during the time that free NE is present in ganglia.

Significance to the Program of the Institute: These studies may add to our knowledge of the physiological mechanisms that regulate individual ganglia of the autonomic nervous system.

Proposed Course of Project: 1) To study mechanism whereby norepinephrine counteracts acetylcholine action. 2) To determine whether frequency response characteristics of the evoked potential are altered by reserpine. 3) To determine whether acetylcholine perfused into the cervical sympathetic ganglion releases ganglionic catecholamines. 4) To determine whether nicotine and other ganglionic stimulants or pharmacologic ganglionic blockade releases catecholamine. 5) To correlate depletion of catecholamines by drugs other than reserpine with changes in the voltage postganglionic evoked potentials. 6) To correlate threshold doses of intracarotid injected acetylcholine to evoke electric discharges in cervical sympathetic ganglia with catecholamine content of ganglia.

Part B included: Yes

Serial No. NHI-208
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Serial No. NHI-209
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: On the Anti-Reserpine Actions of Imipramine (Tofranil).

Principal Investigator: Dr. F. Sulser

Other Investigator: Mr. James Watts

Cooperating Unit: Dr. F. Sulser is a NIH Postdoctoral Research Fellow.

Man Years (Calendar Year 1960):
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: It is generally agreed that imipramine exerts striking effects in certain depressed patients. But in normal animals and man it is a mild stimulant; thus none of screening procedures currently used would suggest this compound to be an antidepressant drug. We have shown that imipramine counteracts the effects of the reserpine-induced syndrome: depression, potentiation of hypnotics, active closure of the eyelids, hypothemia, diarrhea, decrease in motor activity, stimulation of the hypothalamo-pituitary-adrenocortical system etc.

The purpose of these studies is to ascertain the possible mechanism of imipramine using the reserpine induced syndrome of effects as a model.

Methods Employed: Usual chemical and pharmacological procedures.

Major Findings: Certain stressful stimuli diminish or prevent reserpine-induced sedation in intact but not in hypophysectomized animals. However, imipramine still exerts an anti-reserpine action in hypophysectomized animals.

In dogs imipramine prevents the reserpine-induced, centrally mediated parasympathetic salivation (see report on pharmacology of reserpine on the autonomic nervous system) three hours after reserpine, when the submaxillary salivary flow was maximal, imipramine decreased the flow to almost zero. In contrast to Ecolid and atropine, the action of imipramine was not exerted on peripheral parasympathetic ganglia or on the effector organ. Thus on blocking reserpine-induced salivation with imipramine, preganglionic stimulation of the

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chorda tympani still elicited a copious flow of saliva, whereas on blocking with Ecolid or atropine, preganglionic stimulation did not produce salivation. Moreover in acute experiments the response of submaxillary gland to threshold and submaximal stimulation of sectioned chorda tympani was not influenced by imipramine.

These results suggest that imipramine blocks the reserpine-induced parasympathetic response mainly by a central action.

Last year we reported that chlorpromazine also exerts some anti-reserpine action. These results were found to be in error.

Despite reports to the contrary, imipramine administered in various doses did not elevate levels of brain 5HT or norepinephrine. Imipramine given before reserpine to rabbits, rats and mice did not appreciably affect the rate of disappearance of brain 5HT, norepinephrine and dopamine. The change in concentration of intestinal serotonin after reserpine was also the same with and without imipramine pretreatment. It may be concluded that imipramine does not inhibit the pharmacological effects of reserpine by preventing the release of amines stores.

Preliminary studies indicate that a metabolite of imipramine a monomethyl analogue, elicits much more potent anti-reserpine effect than the present compound. This is of considerable interest for imipramine is metabolized rapidly in the body to form an unidentified product present in brain. (See report by Gillette). Small doses (1 mg/kg) of the monomethylated product prevents action of reserpine in rat and dog.

Significance to the Program of the Institute: Using a drug like imipramine as a tool may lead to a better understanding a) of the central actions of reserpine b) of the physiological role of 5HT especially in connection with the impaired balance in the autonomic nervous system.

Proposed Course of Project:

1) We are looking into the possibility that imipramine might act as an anti-serotonin agent in brain and prevent the action of reserpine by competing with the free HT still formed and released to receptor sites.

2) The available metabolites of imipramine will be studied.

Part B included: Yes

Serial No. NHI-209
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

F. Sulser and J. Watts: On the Anti-Reserpine Actions of Imipramine (Tofranil). International Meeting on the Techniques for the Study of Psychotropic Drugs. Bologna 1960, in press.

Serial No. NHI-210
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Evidence that reserpine action is associated with change in brain 5HT; experiments on role of brain 5HT and NE.

Principal Investigators: Dr. E. Costa
Mr. R. Kuntzman
Dr. G. Gessa

Other Investigator: Mrs. C. Hirsch

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: .5
Professional: .4
Other: .1

Project Description:

Objectives: To investigate 1) whether reserpine sedation is associated with change in brain 5HT or with brain NE 2) whether free 5HT acts antagonistically to free NE in brain.

Major Findings: Hess et al. (Pharmacologist 2, 81, 1960), have shown that MMT produces a transient lowering of brain 5HT and a persistent depletion of brain NE. In our experiments mice were given 500 mg/kg (I.P.) of MMT. Brain 5HT declined by 40% in 3 hours and returned to normal values in 10 hours. In contrast brain NE was completely depleted for 24 hours or more. No sedation was seen in these MMT animals. In 13 hours, despite the depletion of NE, decarboxylation and formation of NE in brain still occur. Three kinds of experiments were performed: 1) reserpine in various doses administered 13 hours after MMT lowered brain 5HT and sedation was observed when the amine declined by 50% or more. This result indicates that sedation produced by reserpine coincides with change in brain 5HT and not NE. 2) Reserpine given 10 minutes after 100 mg/kg of MAO inhibitor A-19120 causes exophthalmos, increased motor activity and increased aggressiveness. 3) In contrast, mice given MMT 13 hours before administration of the A-19120 and reserpine showed sedation. These results suggest that free, stabilized NE causes the excitatory state in mice given a MAO inhibitor followed by reserpine, while free stabilized 5HT causes depression.

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Significance to the Program of the Institute: These studies indicate a role of 5HT in brain as a neurohormone of an integrative system that is antagonistic to an adrenergic system.

Proposed Course of Project: These findings will be used as a premise for further investigations on the physiological role of 5HT in brain.

Part B included: No

Serial No. NHI-211
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Development of Stable Reversibly Acting Reserpine Analogues

Principal Investigators: Dr. E. Cuenca
Dr. E. Costa
Mr. R. Kuntzman

Other Investigator: None

Cooperating Unit: Dr. Cuenca is supported by a fellowship from the Institute of International Education

Man Years (Calendar Year 1960):
Total: .4
Professional: .4
Other: None

Project Description: (In collaboration with Ciba Research Laboratories).

Objectives: To develop reserpine analogues that are stable in the intestine. In fact it is difficult to maintain a constant clinical effect with reserpine because the drug is largely and perhaps inconsistently broken down in the gut.

Major Findings: Compounds were made in which the trimethoxy ester linkage was substituted by a methyl ether to form two epimers (SU 8842 and SU 9064). These drugs have a selective action on NE. Small doses cause a persistent decline in brain and peripheral NE. Large doses cause a decline in brain serotonin which lasts about 8 hours. These results suggest the clinical use of these drugs for hypotension and mental illness. For hypotension small doses of drugs should produce chemical sympathectomy without accumulative sedation. For mental diseases larger doses of drugs should elicit a "reversible" tranquilization.

Preliminary findings in psychotics indicate that SU 9064 exerts a reversible effective tranquilizing action.

Significance to the Program of the Institute: From a practical point this study may lead to drugs to be used advantageously in treatment of psychotics and in the therapy of hypertension and peripheral circulatory disorders. From a theoretical point these drugs can be used as tools to detect correlations

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between brain amine content and neurophysiological properties of central autonomic integration.

Proposed Course of Project:

1) Comparison of the dose response curves after chronic and acute administration to evaluate cumulative effects; 2) study of the effects of a selective NE depletion in mechanisms of central autonomic reflectivity; 3) evaluation of the behavioral and EEG effects resulting from a selective depletion and stabilization of high levels of brain 5HT; 4) study of the behavioral effects of morphine in cats completely and selectively depleted of brain NE content.

Part B included: no

Serial No. NHI-212
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Physiological and Biochemical Effects of N-methyl-N-benzyl-2-propynylamine (MO 911) a Non-hydrazine Monoamine Oxidase Inhibitor

Principal Investigator: Dr. S. Spector

Other Investigator: Mrs. Carolynne Hirsch

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: .4
Professional: .2
Other: .2

Project Description:

Objectives: Monoamine oxidase inhibitors used in treatment of depression are generally hydrazides which can exert very toxic effects. The present study is designed to gain some understanding of the biochemical actions of a potent non-hydrazine monoamine oxidase inhibitor.

Major Findings: The monoamine oxidase inhibitors iproniazid, phenylisopropyl hydrazine (JB 516), phenylisobutylhydrazine (JB 835) raise brain levels of serotonin and dopamine rapidly while norepinephrine increases slowly; however, the excitation elicited by the inhibitors is related with the increased brain level of norepinephrine and not with that of serotonin and dopamine.

MO 911, unlike the above mentioned inhibitors, does not contain a hydrazine group and yet inhibits irreversibly MAO. In vitro this compound inhibits brain monoamine oxidase at concentration of 10^{-6} M. In rabbits a single dose causes complete inhibition of the enzyme for 72 hours. Chronic administration of MO 911 to rabbits, similar to the other mentioned inhibitors, elicited excitation which appears to be related with the increased brain levels of norepinephrine but unrelated with brain serotonin and dopamine levels.

Significance to the Program of the Institute: These studies increase our understanding of the mechanisms of action of drugs which are useful in the treatment of various cardiovascular and mental diseases. Furthermore, they may help in determining whether the hydrazine group is responsible for the irreversible damage of CNS caused by irreversible MAOI derived from hydrazines.

Proposed Course of Project: The pharmacological and biochemical studies will be completed as soon as possible.

Part B included: No

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Serial No. NHI-213
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Physiological Role of Catecholamines in Brain

Principal Investigators: Dr. S. Spector
Mr. R. Kuntzman

Other Investigator: Mrs. C. Hirsch

Cooperating Unit: None

Man Years (Calendar Year 1960):
Total: .4
Professional: .3
Other: .1

Project Description:

Objectives: The present report correlates the biochemical and pharmacological effects produced in rabbits by a monoamine oxidase inhibitor given after reserpine.

Major Findings: Monoamine oxidase inhibitors (JB 807 and MO 911) given to rabbits after pretreatment with reserpine antagonizes miosis, belpharospasm and sedation. In contrast to the prompt reserpine reversal caused by amphetamine, the antagonistic action of MAOI occurs 8 to 10 hours after the injection of the drug. Analysis of brain amine concentration shows that serotonin and dopa levels increase rapidly and level off after 2 hours. At this time the rabbits are still strongly sedated. In 10 hours sedation is reversed, brain NE is the only amine whose level is significantly greater than at the 2 hour period. Since binding sites for amines are still impaired by the reserpine the reversal of sedation is presumably caused by the small rise in free norepinephrine. This is the first experiment which definitively indicates that free NE in brain causes behavioral arousal.

Significance to the Program of the Institute: These studies aid the understanding of the role played by norepinephrine, serotonin and dopamine in reserpine induced sedation and prove that reserpine sedation can be counteracted by increases of free brain norepinephrine. As in the case of amphetamine, this antagonistic action reflects a stimulation by NE of brain structures which are in physiological antagonism with the sites of reserpine action.

Proposed course of project: A manuscript on this project will be submitted for publication.

Part B included: No

Serial No. NHI-214
1. Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effect of Barbiturates on Brain Neurohormones

Principal Investigators: Dr. G. L. Gessa
Dr. E. Costa

Other Investigator: Mr. R. Kuntzman

Cooperating Units: None

Man Years (Calendar Year 1960):
Total: .2
Professional: .1
Other: .1

Project Description:

Objectives: To ascertain if, with chemical methods, one could confirm, the data obtained with bioassay, demonstrating as increase of brain 5HT in rats given barbiturates.

Major Findings: Spectrophotofluorimetric analysis of brain of rats given different doses of various types of barbiturates failed to reveal a change in brain 5HT and NE content.

Significance to the Program of the Institute: May add to a better understanding of the mechanism of action of barbiturates.

Proposed Course of the Project: Comparison of bioassay and chemical analysis to ascertain if the discrepancy found is due to interferences in the bioassay of a new biochemical factor. Should this be the case this factor may have some bearing in the mode of action of barbiturates.

Part B included: No

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Serial No. NHI-215
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies on amine oxidation.

Principal Investigator: Herbert Weissbach

Other Investigators: Thomas E. Smith and Jean Renson

Cooperating Units: Dr. E. Maxwell, Dr. B. Witkop, Dr. A. Robertson, NIAMD, NIH

Man Years:
Total: 1.0
Professional: 0.2
Other: 0.8

Project Description:

Monoamine oxidase activity is associated with the mitochondria, but attempts at solubilization and purification of this enzyme have not been very successful. Therefore, information on the mechanism of action of the enzyme, its cofactor requirements, and other characteristics are lacking.

By differential extraction of mitochondria with digitonin, a partial solubilization and 10 fold purification of MAO has been achieved. This preparation has been found to catalyze, as with the mitochondria preparation, the oxidation of N-mono and di-substituted amines. In addition the N-oxides of the N,N-dimethyl derivatives of tryptamine and tyramine have been found to be metabolized by this enzyme. Since the metabolism of these N-oxides is not inhibited under anaerobic conditions, the possibility arises that these compounds or closely related derivatives may be intermediates in the oxidation of amines by MAO. Studies with "labeled" substrates, H_2O^{18} and O_2^{18} are in progress to verify this. Further purification of the enzyme from guinea pig liver will also be attempted, and the metabolism of N-oxides in soluble preparations of MAO from other sources will be studied.

A rapid spectrophotometric assay for MAO based on the rate of disappearance of kynuramine has been developed and this principle extended to studies with snake venom L-amino acid oxidase. Spectrophotometric assays for this latter enzyme have now been developed based on the oxidation of either kynurenine or 3,4-dehydroproline.

It has been postulated that the aldehydes produced by the action of MAO on amines such as tryptamine or serotonin are then metabolized by aldehyde dehydrogenase to the corresponding acids. Experiments with purified indoleacetaldehyde have confirmed this. Since a variety of steroids have been shown to inhibit aldehyde dehydrogenase, under certain conditions, the effect of steroids on the oxidation of indolealdehydes was determined. Inhibition has been observed with several steroid hormones such as progesterone, cortisone and testosterone, all of the above studies have been carried out *in vitro*, and as yet there is no evidence that steroids have a similar effect *in vivo*.

Part B included: Yes

Serial No. NHI-215
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Weissbach, H., Smith, T.E., Daly, J.W., Witkop, B. and Udenfriend, S. A Rapid Spectrophotometric Assay of Monoamine Oxidase Based on the rate of Disappearance of Kynurenine. J. Biol. Chem., 235, 1160 (1960).

2. Weissbach, H., Robertson, A., Witkop, B., and Udenfriend, S. Assay of Snake Venom L-Amino Acid Oxidase. Analytical Biochemistry, in press.

Serial No. NHI-216
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS- NIH
Indiyidual Project Report
Calendar Year 1960

Part A.

Project Title: Amino Acid Decarboxylases

Principal Investigator: Herbert Weissbach

Other Investigators: None

Cooperating Units: Mr. W. Lovenberg, Section of Experimental Therapeutics, NHI

Man Years:
Total: 1.1
Professional: 0.1
Others: 1.0

Project Description:

The marked elevation of a number of urinary amines following the administration of monoamine oxidase inhibition in vivo indicated that many of the natural amino acids are decarboxylated by mammalian tissue. Earlier work in this laboratory had shown that guinea pig kidney contains enzymes that actively decarboxylate 5-hydroxytryptophan (5HTP) and dihydroxyphenylalanine (DOPA). More recently it has been shown that tryptophan, phenylalanine, tyrosine, and histidine as well as several unnatural amino acids are also decarboxylated by guinea pig kidney extracts. The enzyme, 5HTP decarboxylase, has now been purified about 100 fold and the activity toward all the other substrates remains constant throughout the purification. This and other evidence indicates that there exists in animal tissues an aromatic L amino acid decarboxylase which can decarboxylate any of the natural aromatic amino acids. If one expresses the activity of this enzyme towards 5HTP as 100 the other substrates have relative activities as follows: DOPA, 230; Tryptophan, 22, phenylalanine, 10; tyrosine, 3.5 and histidine, 1. Tissues such as brain, liver, and lung also possess the aromatic amino acid decarboxylase.

Alpha methyl DOPA which was originally reported as a competitive inhibitor of DOPA decarboxylase has been found to competitively inhibit all the substrates of the kidney enzyme. Other α-methyl analogues of the aromatic amino acids have also been found to be competitive inhibitors, their effectiveness corresponding to the relative rate of decarboxylation of the parent amino acid. These α-methyl amino acids while being inhibitors of

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the reaction can also serve as substrates, providing evidence that an α-hydrogen is not necessary for the pyridoxal phosphate catalyzed decarboxylation of amino acids.

Further studies with histidine decarboxylase have shown that most cells contain a specific histidine decarboxylase possessing completely different characteristics from the enzyme found in guinea pig kidney. Special attention will be paid to the histidine decarboxylases, since recently Schayer has shown that the activity of a histidine decarboxylase found in the rat stomach is markedly increased during stress conditions. Studies to determine the mechanism of this apparent enzyme induction and the role it plays in vivo are in progress.

Part B included: Yes.

Serial No. NHI-216
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Udenfriend, S., Lovenberg, W., and Weissbach, H. α-amino acid decarboxylase activity in mammalian tissue and its inhibition by α-methyl DOPA. Fed. Proc. 19, 7 (1960).

2. Weissbach, W., Lovenberg, W., and Udenfriend, S. Enzymatic decarboxylation of α-methyl amino acids. Biochem. & Biophys. Res. Comm. 3, 225 (1960).

Serial No. NHI-217
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biosynthesis of the chromopeptide antibiotic actinomycin by Streptomyces antibioticus.

Principal Investigators: Darwin Prockop and Herbert Weissbach

Visiting Investigator: Edward Katz

Cooperating Units: None

Man Years:
Total: 1.0
Professional: 0.8
Other: 0 .2

Project Description:

Studies have been initiated to investigate the metabolic relationship between proline, hydroxyproline and ketoproline found in the various actinomycin molecules. Micro-procedures for the isolation of the different actinomycins were devised. In an initial study with C^{14} labeled L-proline it was found that the actinomycins synthesized by washed suspension of S. antibioticus were isotopically labeled. Moreover, hydroxyproline was found to contain appreciable radioactivity.

The compound, 3,4-dehydroproline was found to influence actinomycin synthesis. It is not certain, however, whether synthesis of two new actinomycins containing this proline analogue occurs or whether the analogue blocks synthesis of the actinomycins containing proline, hydroxyproline and ketoproline. As a result increased synthesis of certain actinomycins containing sarcosine takes place.

Formation of the actinomycin chromophore, 2-amino-4,6-dimethyl-3-phenoxazinone-1,9-dicarboxylic acid, has been achieved using cell-free extracts of S. antibioticus and 3-hydroxy-4-methylanthranilic acid as substrate. In addition, the cell-free system will condense a number of other orthoaminophenols, for example, 3-hydroxykynurenine, 3-hydroxy-4-methylanthranilic acid methylester and 3-hydroxy anthranilic acid to form phenoxzones. Purification and properties of this enzyme system are now under investigation.

Part B included: No.

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Serial No. NHI-218
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biosynthesis and metabolism of melatonin

Principal Investigator: Herbert Weissbach

Other Investigators: Betty Redfield

Cooperating Units: Julius Axelrod, Michael Pare, Irwin Kopin, NIMH, NIH.

Man Years:
Total: 1.0
Professional: 0.6
Others: 0.4

Project Description:

The investigation was undertaken to determine how melatonin (N-acetyl-5-methoxy tryptamine), a compound present in high concentration in the pineal gland and known to be a potent lightening agent in the frog skin is synthesized and metabolized in the body. It has been possible to show the presence of an enzyme in pineal tissue which can O-methylate N-acetylserotonin and other 5-hydroxy indoles. The enzyme has been purified 25 fold and shows an absolute requirement for S-adenosylmethionine (AMe). The first reaction in the biosynthetic pathway involves the acetylation of serotonin to N-acetylserotonin. This reaction has been studied both in mammalian liver and pineal extracts. The enzyme requires acetylcoenzyme A as an acetyl-donor and appears to be able to acetylate a variety of naturally occurring amines such as tryptamine, tyramine, phenylethylamine, and histamine. Further purification is required to determine whether one enzyme is responsible for these reactions. By incubating acetylcoenzyme A (or a system to generate this coenzyme), S-adenosylmethionine and serotonin with pineal extracts it was possible to synthesize melatonin from serotonin. The pathway appears to be as follows:

$$\text{serotonin} \xrightarrow{\text{AcCoA}} \text{N-acetylserotonin} \xrightarrow{\text{AMe}} \text{melatonin}$$

The major in vivo metabolite of melatonin appears to be 6-hydroxymelatonin which is excreted as a glucuronide conjugate. The hydroxylation of the pineal hormone is catalyzed by a microsomal enzyme which is known to hydroxylate other indoles in the 6 position. It is hoped to investigate this latter reaction in more detail in the future.

Part B included: Yes

Serial No. NHI-218
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Axelrod, J., and Weissbach, H.: Enzymatic O-methylation of N-acetylserotonin to melatonin. Science. In press.

Serial No. NHI-219
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Epinephrine, Norepinephrine and Related Compounds.

Principal Investigator: Sidney Udenfriend

Other Investigators: Cyrus R. Creveling

Cooperating Units: Dr. B. Witkop, Laboratory of Chemistry, NIAMB and Dr. J.R. Crout, Section of Experimental Therapeutics, NHI

Man Years (calendar year 1960):
Total: 1.3
Professional: 0.3
Other: 1.0

Project Description:

Studies on the biogenesis and degradative metabolism of catechol amines.

Norepinephrine (NE) is metabolized largely by two alternate enzyme systems: Catechol-o-methyl transferase (COMT) and Monoamine oxidase (MAO). The present study was undertaken to determine which of these enzymes is of greater significance in the degradation of NE in the brain and heart of the rat. The MAO activity, in vitro, was 3.5 and 5 times greater than COMT activity in the brain and heart respectively. In the liver COMT activity was 15 times greater than MAO activity. A significant increase in the NE content of rat brain and heart was produced by intraperitoneal administration of four different MAO inhibitors. A similar administration of pyrogallol, a COMT inhibitor, failed to produce an increase even after 18 hours of enzyme inhibition. NE was injected intravenously into rats, and the rate of NE disappearance from the plasma and the heart measured. In rats pretreated with iproniazid, there was a marked increase in the NE concentration in the heart relative to the plasma; clearance of NE from the plasma was only slightly prolonged. Rats pretreated with pyrogallol demonstrated a markedly decreased plasma clearance, indicating a severe impairment of their ability to metabolize circulating NE. The ratio of the heart/plasma concentration of the amine with the COMT inhibitor was unity, suggesting that the amine content of the heart was only a reflection of the plasma level. These experiments show that endogenous NE accumulates

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in the heart and brain when MAO is inhibited; exogenous NE accumulated in the heart under the same conditions. Neither of these effects is produced by a COMT inhibitor. This suggests that oxidative deamination is more significant in the metabolism of NE in the brain and heart of the rat.

The hydroxylation of dopamine to NE was previously demonstrated in brain and adrenal medulla. The formation of NE is difficult to measure and a new technique was developed to measure the hydroxylase activity. The β-hydroxylation of tyramine was demonstrated in brain, adrenal medulla slices and in purified tissue fractions. The cofactor requirements for the hydroxylation of tyramine with a partically purified enzyme were shown to be ascorbic acid, fumaric acid and ATP; these same cofactors have been previously shown to be required for the hydroxylation of dopamine by other workers. Furthermore dopamine was shown to be a competetive inhibitor of tyramine hydroxylation. The hydroxylation of tyramine is now used to measure the β-hydroxylase activity.

Other possible substrates are being investigated primarily phenylethylamine and m-tyramine.

The mechanism of the β-hydroxylation is being investigated with tritium labeled dopamine and tyramine. The present hypothesis postulates an (enzymatically catalyzed) activation step followed by the hydroxylation of the activated β-position. The exchange of the tritium on the β-position with the incubation media is now being studied as a possible approach to this problem.

Part B included Yes

Serial No. NHI-219
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Pisano, J.J., Creveling, C.R. and Udenfriend, S.: Enzymic conversion of p-tyramine to p-hydroxylphenylethanolamine (Norsynephrine). Biochim. et Biophys. Acta, 43: 566-568, 1960.

Serial No. NHI-220
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Biosynthesis of Choline

Principal Investigator: Kenneth D. Gibson

Other Investigators: Jean D. Wilson and S. Udenfriend

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.7
Professional: 1.7
Other:

Project Description:

Although much is known about the enzymic degradation of the humoral agent acetyl choline we know little about its overall synthesis except what has been obtained from experiments in intact animals. Previous investigations in vivo have shown that choline is synthesized by methylation of ethanolamine. It was generally believed that two of the methyl groups are derived from the one carbon fragment pool, while the third is transferred from methionine. However, our studies indicate that the methionine methyl group is the sole precursor of the choline methyl group in the rat liver and that the conversion of one carbon fragments to the methyl group of choline is preceded by their incorporation into methionine. Furthermore, free ethanolamine and phosphoethanolamine do not appear to be intermediates in this conversion. In both slices and homogenates ethanolamine C^{14} appeared earliest as the ethanolamine of phospholipid. In fractionated homogenates of rat liver, the microsomes contained most of the activity for the synthesis of phospholipid ethanolamine from serine-C^{14}.

Homogenates of rat liver incorporate C^{14} from S-adenosyl methionine-CH_3-C^{14} into phospholipid choline and to a much lesser extent into phosphocholine. The activity is entirely associated with subcellular particles, and incorporation proceeds with no other additions.

Radioactive monomethylethanolamine and dimethylethanolamine have been identified as constituents of the phospholipid of microsomes incubated with S-adenosylmethionine-C^{14}. The incorporation into dimethylethanolamine is consistent with its being a precursor of choline.

Lecithin containing radioactive choline has been isolated from a similar incubation mixture.

These results support the hypothesis that lecithin can be synthesized by the transfer of methyl groups to phosphatidyl ethanolamine. By the introduction of quaternary ammonium groups, the conversion of cephaline to lecithin could alter profoundly the charge on a lipid membrane, and thereby change its structure of permeability. Such an alteration may play a part in determining the lamellar structure of the endoplasmic reticulum, or in some of the functions of intracellular transport and conduction which have been suggested for this component of the cytoplasm. It is significant in this connection that in rat liver both the methylation of phosphatidyl ethanolamine and the decarboxylation of phosphatidyl serine occur primarily in microsomes, which recent evidence has shown to be identical with the granular components of the endoplasmic reticulum.

Dr. Gibson and Dr. Wilson have both returned to their respective departments and this project has been discontinued temporarily.

Part B included: Yes

Serial No. NHI-220

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Wilson, J.D., Gibson, K.D. and Udenfriend, S.: Studies on the precursors of the methyl groups of choline in rat liver. J. Biol. Chem. 235: 3213-3217, 1960.

2. Wilson, J.D., Gibson, K.D. and Udenfriend, S.: Studies on the conversion in vitro of serine to ethanolamine by rat liver and brain. J. Biol. Chem., 235: 1960, in press.

3. Gibson, K.D., Wilson, J.D. and Udenfriend, S.: The enzymatic conversion of phospholipid ethanolamine to phospholipid choline in rat liver. J. Biol. Chem., in press.

Serial No. NHI-221
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Hydroxyproline and Collagen.

Principal Investigator: Darwin J. Prockop

Other Investigators: Beverly Peterkofsky

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.7
Professional: 1.0
Other: 0.7

Project Description:

Objectives. To study the pathways for the synthesis and degradation of collagen.

Methods Employed. A major emphasis is placed on quantitative and radioactive measurements of hydroxyproline, since this imino acid is found almost exclusively in collagen.

Major Findings. 1. Measurements of hydroxyproline-C^{14} in the urine of rats which had previously been injected with proline-C^{14} has revealed the presence of forms of collagen which are rapidly synthesized and degraded. The studies confirmed earlier reports that body collagen, taken as a whole, is metabolically inert, but they demonstrated for the first time that there are small pools of collagen which enter into the same "dynamic state" as other tissue constituents. Young rats were found to contain much more of this metabolically active collagen than old.

2. Preliminary in vivo studies in the chick embryo have shown that collagen is synthesized by the same microsomal pathway which has been suggested for the synthesis of other proteins.

3. We appear to have been successful in preliminary attempts to obtain an in vitro system from the chick embryo which will synthesize collagen.

Proposed Course of Project. 1. Further radioactive studies in rats are underway to measure the turnover of various forms of collagen

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in the rat. The studies should help to explain the aging of collagen as animals grow older.

2. The in vivo system for collagen synthesis which we have studied in chick embryos should be ideal for studying the formation of hydroxyproline from proline, since this hydroxylation is thought to occur only in the formation of the collagen molecule. Compounds which might be intermediates in the formation of hydroxyproline are being prepared by Dr. Bernard Witkop and will be tested in this system. An attempt will also be made to verify an earlier report from this laboratory that there is a small incorporation of free hydroxyproline into collagen.

3. Attempts will be made to verify the in vitro system for collagen synthesis. This should provide a valuable tool in studying collagen synthesis in detail.

Part B included Yes

Serial No. NHI-221
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Prockop, D.J. and Udenfriend, S.: A specific method for the analysis of hydroxyproline in urine and tissues. Analytical Biochem., in press.

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Serial No. NHI-222
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biosynthesis of γ-Guanidinobutyric Acid

Principal Investigator: J. J. Pisano

Other Investigators: David Abraham

Cooperating Units: None

Man Years:
Total: 0.10
Professional: 0.05
Other: 0.05

Project Description:

Since the last report, brain transamidinase has been purified and the transamidination of arginine with γ-aminobutyric acid in brain has been proven. This terminates research on this problem.

Part B included: No.

556

Serial No. NHI-223
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Discovery, Biosynthesis and Function of Synephrine and Norsynephrine.

Principal Investigator: John J. Pisano

Other Investigators: J.A. Oates, A. Karmen, A. Sjoerdsma and C. Creveling

Cooperating Units: Section on Experimental Therapeutics (Drs. Oates and Sjoerdsma) and Laboratory of Technical Development (Dr. Karmen)

Man Years (calendar year 1960):
Total: 0.8
Professional: 0.8
Other:

Project Description:

During the development of an assay for the metanephrines another unknown substance was observed with similar chemical properties. This fact recalled earlier observations in this laboratory on the possible occurrence in urine of an unknown amine and lead to the discovery of synephrine and norsynephrine in human urine. These amines are identical in structure to adrenaline and noradrenaline, respectively, except that they lack the m hydroxy group, and although they have been on the chemist's shelf for many years, they were not known to be present in human urine. An assay has been developed (based on the metanephrine method) for the quantitative determination of the combined synephrines and their metabolic end-product, p-hydroxymandelic acid, in urine. In 12 normal patients the average excretion of the synephrines was 650 μg/day and of the metanephrines 400 μg/day. Administration of monoamine oxidase inhibitors caused a 3 fold increase. Preliminary data on the infusion of tyramine substantiates the earlier in vitro findings that this amine is the precursor of the synephrines. The in vitro discovery that tyramine is actively hydroxylated by adrenal medulla and brain slices has been extended to show that the enzyme which catalyzes this reaction is the same enzyme which catalyzes the hydroxylation of dopamine to noradrenaline namely, dopamine-β-oxidase. Measurement of the activity of this important enzyme was hitherto difficult because of the interference of dopamine in the noradrenaline assay. The conversion of tyramine to norsynephrine, on the other hand, is easy to follow since tyramine does not interfere in the norsynephrine assay. Other amines are currently being investigated for possible activity with this enzyme.

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Current and future research is directed towards the discovery of the physiological function of the synephrines. Their close structural relationship to adrenaline and noradrenaline and the fact that their biosynthesis involves the same enzyme suggests a function related to the catecholamines. Experiments are planned for the long term administration of tyramine to animals to determine if this will effect a depletion of the catecholamines through competition with DOPA for dopamine-β-oxidase. This may lead to some striking physiological changes of importance in understanding the physiological role of adrenaline and noradrenaline as well as the synephrines.

Part B included Yes

Serial No. NHI-223
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Pisano, J.J., Creveling, C.R. and Udenfriend, S.: Enzymic conversion of p-tyramine to p-hydroxyphenylethanolamine (norsynephrine). Biochim. et Biophys. Acta, 43: 566-568, 1960.

2. Pisano, J.J., Oates, J.A., Karmen, A., Sjoerdsma, A. and Udenfriend, S.: Identification of p-hydroxy-α-(methylaminomethyl)benzyl alcohol in human urine. J. Biol. Chem., in press.

Serial No. NHI-224
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Chemical Determination of the Metabolites of Epinephrine and Norepinephrine.

Principal Investigator: John J. Pisano

Other Investigators: David Abraham

Cooperating Units: Richard Crout and Doris Watts, Section on Experimental Therapeutics

Man Years (calendar year 1960):
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

With the development of a practical method for the determination of the combined metanephrines in urine, attention was next directed to the development of an assay for the other major metabolite of the catecholamines, 3-methoxy-4-hydroxymandelic acid. This problem has been solved and a procedure has been perfected for the quantitative determination of the acid metabolite in normal urine as well as in patients with pheochromocytoma and certain other neural tumors (ganglioneuromas and neuroblastomas). Application of the methods to the determination of the urinary excretion of catecholamine metabolites has revealed that in normal individuals (and in hypertensive patients) the combined excretion of metanephrines and of 3-methoxy-4-hydroxymandelic acid was 0.60 mg/day and 4.0 mg/day respectively, whereas the excretion of the catecholamines was only 0.03 mg/day. In 23 patients with pheochromocytoma, the excretions ranged from 1.6 - 41 mg/day for the metanephrines, 4.4 - 530 mg/day for 3-methoxy-4-hydroxymandelic acid and 0.2 - 4.3 for the catecholamines. These findings represent the most thorough analysis of the urinary excretion of the catecholamines and metabolites in pheochromocytoma. Every new hypertensive patient at the NIH is now screened for pheochromocytoma by the methods described above and it is believed that the procedures will be an important aid in the diagnosis of pheochromocytoma.

Current research is directed to the modification of the metanephrines method to increase the sensitivity of the assay and thus make it applicable to the low levels found in tissues and in normal urine.

Serial No. NHI-224

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Pisano, J.J.: A simple analysis for normetanephrine and metanephrine in urine. Clin. Chem. Acta, 5: 406-414, 1960.

2. Crout, R.J., Pisano, J.J. and Sjoerdsma, A.: Urinary excretion of catecholamines and their metabolites in pheochromocytoma. Am. Heart Journal, in press.

Serial No. NHI-225
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Discovery and Metabolism of Homocarnosine

Principal Investigators: John J. Pisano
J. D. Wilson

Other Investigators: David Abraham

Cooperating Units: Dr. L. A. Cohen, Laboratory of Chemistry, NIAMD

Man Years:
Total: 1.0
Professional: 0.6
Other: 0.4

Project Description:

The peptide isolated from brain (described in the previous report) has been positively identified as γ-aminobutyrylhistidine (homocarnosine). This substance is found exclusively in brain and spinal cord but not in peripheral nerves or other tissues. Unlike γ-aminobutyric acid, it is found in high concentrations in spinal cord; white matter contains more than grey matter. Human, monkey and cow brains contain the highest levels (20 μmoles/100g) and dog and hog contain the lowest (0.3 μmole/100 g). An assay has been developed for the measurement of homocarnosine based on ion exchange chromatography. By feeding γ-aminobutyric acid to rats it was possible to show the presence of homocarnosine in muscle where it does not normally occur. Rats receive little or no γ-aminobutyric acid in their diet and by feeding it we were able to detect it in muscle where it condensed with histidine to form homocarnosine. This finding corroberates the work of others where it was shown that the carnosine synthetase of muscle can utilize γ-aminobutyric acid.

Current and future research is directed towards understanding the function of homocarnosine. Studies are underway or planned to determine the turnover, biosynthesis and degradation of the peptide. Also, a study will be made to relate the biochemistry of homocarnosine with brain activity. Homocarnosine has been detected in human urine and since it is formed only in brain, the exciting possibility is evident that alterations of this compound in urine may reflect cerebral function or disfunction. It is planned to compare the urinary excretion of this peptide in normals and in individuals with a variety of central nervous system disorders.

Part B included: Yes

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Serial No. NHI-225
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Pisano, J.J., Wilson, J.D. and Udenfriend, S.: γ-Aminobutyric acid as a possible precursor of other physiologic substances. Inhibition in the nervous system and γ-aminobutyric acid. C. Roberts Editor, Pergamon Press, New York, 1960.

2. Pisano, J.J., Wilson, J.D., Cohen, L., Abraham, D. and Udenfriend, S.: Isolation of γ-aminobutyrilhistidine (homocarnosine) from brain. J. Biol. Chem., in press.

Serial No. NHI-226
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Hydroxylation of Aromatic Compounds

Principal Investigator: Sidney M. Hess

Other Investigators: Charles A. Chidsey

Cooperating Units: None

Man Years
Total: 1.5
Professional: 1.5
Other:

Project Description:

Efforts were continued to determine the mechanism of aromatic hydroxylation in vivo. To find alternate pathways of the normal p-hydroxylation of phenylalanine toad liver metabolism of the latter compound was investigated. It was learned that lack of tyrosine formation in toad liver incubation mixtures was attributable to the relatively low rate of metabolism. Lactic dehydrogenase activity in toad liver is four times slower than the activity found in rat liver. No other hydroxylated products of phenylalanine were detected in this system.

Using rabbit liver microsomes no hydroxylation of phenylethylamine to form o, or m,-tyramine was found. A very small trace of p-tyramine was found in one experiment (less than 0.13 μM/g/hr.).

Efforts to increase the activity of the microsomal hydroxylating enzyme by pretreatment with 3-methyl cholanthrene or 3,4-benzpyrene were non-productive. Ortho hydroxylation of phenylacetic acid was not found in rabbit liver incubation systems or in urine collected over a two day period.

Folic acid deficient chicks were shown to exhibit essentially normal formation of tyrosine from phenylalanine. On the other hand the addition of methotrexate to incubation beakers containing rabbit liver microsomes and acetanilide caused a variable 20-100% increase in the amount of p-hydroxyacetanilide formed.

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We were not able to repeat the reported solubilization of the rabbit liver microsomal aniline hydroxylation enzyme using the solvent extraction system of methyl alcohol-acetone-ether or the second system employing Okinawan snake venom.

Using tritiated phenylalanine and the two separate soluble components (rat liver enzyme and sheep liver enzyme) required for the formation of tyrosine hydroxylation was shown to occur, but when only the hydroxylating enzyme, rat-liver component, was employed no activation of phenylalanine could be detected by the presence of tritium in the supernate of the incubation beaker. The counterpart of this experiment employing T_2O and phenylalanine gave the same results -- no preactivation was found of the phenylalanine prior to the actual hydroxylating step.

α-Methylphenylalanine did not inhibit the hydroxylation of phenylalanine.

Part B included: No.

Serial No. NHI-227
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Uptake of α-Amino Acids by Mammalian Cells

Principal Investigator: Gordon Guroff

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.0
Professional: 1.0
Other: None

Project Description:

An elucidation of the process by which amino acids enter cells is of importance in nutritional, pharmacological, and clinical investigations in addition to being of interest in a general sense. The further metabolism of a molecule is determined to a great extent by the rate and specificity of its uptake and the interactions between it and other circulating molecules.

A study of the uptake of tyrosine by brain and other organs in vivo has revealed that elevations in blood levels, produced by intraperitoneal injection, give rise to elevations in brain tyrosine. Within 60 minutes the brain level exceeds the plasma level. The entrance of tyrosine has great stereospecificity, D-tyrosine being almost completely excluded. A small, but significant racemization accounts for the small amounts of tyrosine entering brain following intraperitoneal administration of D-tyrosine. Other amino acids, when present in the blood stream, depress tyrosine uptake. Tryptophan, leucine, isoleucine, valine and others are quite active in this regard while glutamic acid, argenine and others are not. Certain unnatural amino acids such as norvaline and p-fluorophenylalanine also depress brain uptake. An investigation of leg muscle uptake reveals that, although the rate of penetration of tyrosine is comparable to that of brain, the stereospecificity of uptake and the competition by other amino acids is either completely lacking or is much less evident.

It has been shown through a study of the uptake of tyrosine by diaphragm in vitro that tyrosine enters the diaphragm cell by a process of diffusion. The entry is proportional to the external concentration and

unaffected by the ionic composition of the medium, the pH, the presence of external substrates, or the presence of related amino acids. The temperature coefficient for uptake is 1.3-1.5 and the uptake is the same in boiled tissue as in metabolically active preparations. A slight stereospecificity can be shown. Kinetic and isotopic data indicate that the free tyrosine of the tissue is not in equilibrium with tyrosine added in vitro.

The uptake of tyrosine by brain slices resembles the uptake by brain in vivo in certain particulars. The uptake is rapid, concentrative, and can be stimulated by glucose and other carbohydrates and by divalent cations. The concentrative component of the uptake can be abolished by boiling the tissue, by incubating the tissue in the absence of oxygen, or through the use of any of a number of metabolic poisons. The amino acids, which depress or eliminate tyrosine uptake in vivo are able to eliminate concentration of tyrosine by the tissue in vitro. Amino acids ineffective in vivo are ineffective in vitro. On the other hand, molecules which do not enter brain in vivo, for example, p-hydroxyphenylacetic acid, do diffuse into brain slices. Further, D-tyrosine which is excluded from brain in vivo enters the brain slice and is concentrated by the slice to the same extent as the L-isomer. Slices of other tissues, some of which apparently concentrate tyrosine slightly, do not respond to metabolic poisons or to the stimulatory effects of glucose. A tentative explanation for the findings is that the in vitro system represents one portion of the total tyrosine uptake mechanism found in brain in vivo. This portion possesses the concentrating powers but does not prevent the entrance of molecules normally excluded from the brain in vivo.

At present the major effort of the in vivo studies is to define the nature of the competitive effect of certain molecules on tyrosine uptake by brain. It will be of interest to know if a.) molecules other than amino acids can compete; b) if the entry of the competitor is mandatory, c) if the total of the competitor and of tyrosine entering is a constant value at any level of competition, and d) if any predictions can be made as to the structural or physical requirements for an effective competitor. Certain phases of the in vitro system are being more closely studied. Of most interest is the mechanism of the various stimulators. It is desirable to know whether these carbohydrates are acting through their metabolic contributions or by other means. It is hoped to shed light on the mechanism of the uptake and to extend the investigations to other tissues, including tumors, and to other classes of nutrients.

Part B included: Yes.

Serial No. NHI-227
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Chirigos, M. A., Greengard, P. and Udenfriend, S.: Uptake of Tyrosine by Rat Brain in vivo. J. Biol. Chem., 235: 2075-2079, 1960.

2. Guroff, G. and Udenfriend, S.: The Uptake of Tyrosine by Isolated Rat Diaphragm. J. Biol. Chem., in press.

3. Guroff, G. and Udenfriend, S.: The Failure of Insulin to Effect the Transport of Tyrosine in the Isolated Rat Diaphragm. Biochim. et Biophys. Acta, in press.

Serial No. NHI-228
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Metabolism of Amines; Decarboxylation.

Principal Investigator: Sidney M. Hess

Other Investigators: M. Ozaki, Robert Connamacher and Charles A. Chidsey

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.5
Professional: 1.0
Other: 0.5

Project Description:

Decarboxylation of 3,4-dihydroxyphenylalanine, (DOPA), has been shown to be inhibited by α-methyl DOPA and α-methylmetatyrosine. Both of these compounds were shown to lower brain serotonin (5HT), norepinephrine (NE) and DOPAmine in guinea pigs. The first to return to normal was the 5HT, after about 18 hrs., this was followed by the DOPAmine which returned to normal in about 30 hrs., but the NE remained at depressed levels for days longer both in the brain as well as in the heart. The depletion of 5HT and probably DOPAmine in the brain parallels closely the inactivation of the decarboxylase, but the depletion and persistent low levels of NE may not be entirely due to decarboxylation inhibition.

The effects were produced by L,α-methyl DOPA but not by the D-isomer 2,3-dihydroxy-α-methylphenylalanine—another potent decarboxylase inhibitor was found to be inactive with respect to all amines.

Dr. Chidsey produced thyrotoxic rats by daily administration of L-thyroxine. These rats exhibited elevated O_2 consumption, and increased heart weight but contrary to earlier reports, no increase of heart NE, or DOPAmine. Administration of reserpine to thyrotoxic rats caused a depletion of heart NE but no change on the elevated metabolic state measured by O_2 consumption.

Part B included No

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Serial No. NHI-229
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The influence of various solutes on the movement of water across a living membrane (toad bladder) in the presence and absence of vasopressin.

Principal Investigators: Laurence Earley
Jack Orloff

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 5/12
Professional: 5/12
Other: 0

Patient Days: 0

Project Description -

Objectives: To obtain further evidence for the independence of water and solute movements across living membranes and to study the influence of permeating solute on water movement.

Progress during the past year: Isolated urinary bladder halves of the toad Bufo Marinus have been suspended as closed sacs containing various test solutions. The sacs are bathed by a toad Ringer solution to which may be added vasopressin or various concentrations of other solutes. Net water movements are measured by changes in weight of the bladder sac. Net solute movements are estimated by direct analysis. Water moves across the bladder wall at a rate dependent upon the magnitude of the osmotic gradient imposed. The direction of the osmotic gradient in relation to the two sides of the membrane does not influence the rate of water movement, nor does the nature of the solute so long as the latter does not penetrate the membrane. Net water movement is increased approximately fifty fold by the addition of vasopressin to the media bathing the serosal surface of the bladder. This response to vasopressin occurred in sodium free media, indicating that active sodium transport is not a requisite to the action of antidiuretic hormone on water permeability. In the absence of vasopressin net water movements in response to osmotic gradients established by either sodium chloride, mannitol, or urea are approximately the same. This is not the case in the presence of vasopressin. Net water losses from hypotonic solutions of urea within the bladder sac are greater than from solutions of mannitol or sodium chloride of similar concentration. Net water gain into hypertonic solutions of urea are less than into solutions of mannitol or

sodium chloride. Although urea penetrates the bladder wall at very high rates in the presence of vasopressin, hypotonic solutions of urea within the bladder may be concentrated (during one hour) to equal the total concentration of Ringer plus urea outside the bladder. Urea and water appear to move across the bladder wall largely independent of each other, their relative rates of movement being dependent upon their respective gradients.

Direction of current research: These studies are to be continued in an effort to obtain further information concerning the movements of urea and water under different conditions. It is anticipated that this system may provide a means for study of the mechanisms whereby vasopressin increases membrane permeability and the factors which influence this action.

Major findings of significance: None.

Serial No. NHI-230
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: A Study of Renal Phosphorylase Activity

Principal Investigators: Joseph S. Handler
Jack Orloff

Other Investigator: Maurice B. Burg

Cooperating Units: None

Man Years - Patient Days: 0
Total: 5/12
Professional: 5/12
Other: 0

Project Description -

Objectives: A number of enzyme systems affecting phosphorylase metabolism are present in the kidney. However the system, as well as factors affecting it, has not been characterized in any detail. The present study was designed to characterize renal phosphorylase with particular emphasis on the effects of hormonal agents.

Progress during the past year: Phosphorylase activity was assayed using the method devised for estimation of liver and muscle phosphorylase. Kidneys of normal, fed, hydrated, male Sprague-Dawley rats were used in all studies. Tissue slices were prepared in the standard manner; the slices were incubated in oxygenated Krebs-bicarbonate medium. Phosphorylase activity was determined in the presence of sodium fluoride and EDTA. A modified assay system was developed in order to estimate the activity of phosphorylase in renal homogenates.

Major findings of significance: Renal phosphorylase appears to resemble that characteristic of muscle, rather than of liver; that is, the inactive form is activated by the addition of adenylic acid. In slices, a 40-50% fall in activity occurs in the course of 2 hour incubation. This pattern is unaffected by the addition of vasopressin to the incubation medium. Studies of the behavior of renal homogenates have revealed that activity disappears rapidly on incubation in water. In view of the fact that the inactivation can be inhibited by the addition of fluoride and EDTA this may indicate that the effect is due to the presence of a so-called

phosphorylase rupturing enzyme.

Proposed course: Further characterization of the system in both kidney slices and homogenates is being undertaken prior to examination of the effects of a variety of hormones.

Serial No. NHI-231
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Renal Excretion of Phosphate in the Dog

Principal Investigators: Joseph S. Handler
Jack Orloff

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 4/12 Patient Days: 0
Professional: 4/12
Other: 0

Project Description -

Objectives: There are several reports in the literature suggesting that phosphate is secreted by the renal tubules of mammals. Although none of these studies contain conclusive evidence of secretion, they are now frequently quoted as proving phosphate secretion in mammalian kidneys. It is the purpose of this study to try to demonstrate secretion in a more conclusive manner, i.e. - considerably greater clearance of phosphate than the simultaneous clearance of inulin, under reasonably steady state conditions.

Major Findings: Mongrel dogs were studied under pentobarbital anesthesia, which has been chosen because of its reported enhancement of renal phosphate excretion. Sodium phosphate, pH 7.3-7.4, in an isotonic or slightly hypertonic solution, was infused at a controlled rate so that clearances were measured when serum phosphate concentrations varied from less than 5 to over 10 or 15 mM/L. in each dog. The dogs were divided into groups of two to seven dogs each, and the members of each group studied under similar conditions and after similar preparation. The resulting combination of variables in different groups were as follows: a group with no previous preparation, a group made hyperparathyroid by the administration of exogenous parathyroid extract for three days prior to study and on the day of study (the resulting hyperparathyroid activity was checked by measuring serum calcium concentration at the start of the clearance experiment), a group made similarly hyperparathyroid and studied under a mannitol diuresis, a group with normal parathyroid function studied under a mannitol diuresis, and a last group loaded with sodium phosphate intravenously for three days prior

Part B included - No.

to the experiment. In approximately half the dogs in each group urine was collected from the bladder, and in others urine was collected from each ureter and clearances estimated separately for each kidney. In the latter situation, an inflatable cuff was placed around one renal artery and renal blood flow reduced on that side, so that clearances at two rates of glomerular filtration were obtained in each of these dogs.

Although ratios of the clearance of phosphate to the clearance of inulin approaching or equal to 1.0 were occasionally obtained, the ratios were generally less than 1.0. The data suggest a maximum rate of tubular reabsorption of filtered phosphate. No evidence for net secretion has been obtained thus far.

Projected studies: There is some questionable evidence that renal tubular phosphate metabolism may be affected by sodium depletion. It is planned to deplete two or three dogs of sodium by dietary restriction plus a diuretic a few days before study, and then to examine clearances as in the other dogs.

Serial No. NHI-232
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Mechanism of Urinary Acidification

Principal Investigators: Robert W. Berliner
Georg Peters

Other Investigators: Herbert Lubowitz
Bruce F. Bower

Cooperating Units: None

Man Years: Patient Days: 0
Total:
Professional:
Other:

Project Description -

Objectives: It has in recent years been assumed that bicarbonate reabsorption in both proximal and distal tubules is effected by an exchange of hydrogen ions for sodium ions with the resultant formation of carbonic acid in the tubule lumen. It has been further assumed that the carbonic acid then breaks down to CO_2 and water and that the CO_2 escapes by diffusion. To provide for a rate of formation of CO_2 equal to the rate of bicarbonate reabsorption 1) a considerable concentration of carbonic acid is required or 2) the reaction must be catalyzed by carbonic anhydrase. If the first alternative is correct, the pH of the fluid would, in situ, be about one unit lower than that achieved when CO_2 and H_2CO_3 had reached equilibrium (as would be the case in all measurements which have been made since these involved removal or isolation of the samples). If the second alternative is correct, the response of pH to carbonic anhydrase inhibition would be a lowering, i.e. the reverse of that which has always been assumed to occur.

The investigation of these possibilities requires determination of the pH of proximal tubule fluid in situ by the insertion of appropriate microelectrodes.

Direction of current research: Development of a pH-sensitive microelectrode for use in the study of the mechanism of urinary acidification.

Major findings of significance: Preliminary experiments with the few workable microelectrodes that have been developed show no evidence of acidification in the proximal tubule of necturus maculosa nor evidence suggesting that the dynamic pH differs from the equilibrium pH.

Part B included: No.

Serial No. NHI-233

1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Measurement of Renal Medullary Blood Flow.

Principal Investigators: Bruce Bower
Robert W. Berliner

Other Investigator: Herbert Lubowitz

Man Years:
Total:
Professional:
Other:

Project Description -

Objectives: To explore various approaches towards measurement of renal medullary blood flow in the experimental situation.

Progress During Past Year: The mathematical approaches to the measurement of renal medullary blood flow with a variety of ideal indicators has been studied and the assumptions inherent in these measurements defined. It has, on the basis of these studies, seemed advisable to use either indicators to which the vascular bed is impermeable or freely diffusible gases. Preliminary studies have been undertaken along both of these lines.

The use of changes in electrical impedance measurements of the medulla following injections of dextran-water solutions has been investigated. At the frequency selected (1000 kc) the observed changes were not sufficient to justify further studies.

Several renal artery injections of I^{131} albumin and colloidal Au^{198} have been performed with continuous withdrawal from the ipsilateral vein and simultaneous sampling from the aorta to correct for recirculation. Analysis of the resultant curves has been undertaken to determine the volumes of distribution and slope constants of the various compartments of the kidney so studied. The relatively slow blood flow and large blood volume of the medulla may separate its component curve in time from that of the cortex and other relatively rapidly cleared vascular spaces. Further studies will be required to substantiate this concept. In this regard colloidal Au^{198} has been most satisfactory since removal of these particles by the reticulo-endothelial elements of the spleen and liver minimizes recirculation.

A system for the potentiometric measurement of freely diffusible hydrogen gas has been constructed and its sensitivity to small changes in

concentration demonstrated. A platinum-platinum black electrode is used with a standard calomel-kcl electrode serving as reference. Measurements have not as yet been undertaken *in vivo*.

Direction of current research:

Further exploration of use of both hydrogen and colloidal Au^{198} as indicators for measurement of renal medullary blood flow.

Major findings of significance: None.

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Serial No. NHI-234 (c)
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The antidiuretic effect of the chlorothiazide derivatives in diabetes insipidus.

Principal Investigator: Jack Orloff

Other Investigator: Laurence Earley

Cooperating Units: None

Man Years:
Total: 6/12
Professional: 6/12
Other: 0

Patient Days: 200

Project Description -

Objectives: It has been reported elsewhere that the chlorothiazide derivatives diminish urine flow and increase urine osmolality in patients and experimental animals with diabetes insipidus. Evidence in both dog and man indicates that these agents interfere with sodium reabsorption at the diluting site within the renal tubule. Although this effect readily accounts for the observed increase in urine osmolality in diabetes insipidus it does not afford an explanation for the associated reduction in urine flow which results from administration of the drug. The present study was undertaken to investigate the mechanisms whereby the thiazide derivatives reduce urine flow in diabetes insipidus and to evaluate its usefulness in the clinical management of vasopressin resistant diabetes insipidus.

Progress: Chlorothiazide or hydrochlorothiazide has been administered to four patients with vasopressin resistant diabetes insipidus. In all subjects a reduction in urine volume and an increase in urine osmolality occurred within two days. The magnitude of the response was greater in subjects maintained on a low sodium diet and persisted in these for a variable period of time when the drug was withdrawn. Persistence of the antidiuretic effect was not observed in patients maintained on a high sodium diet nor was the initial response as great.

The antidiuresis was associated with a loss of body sodium and a reduction in body weight. Changes in filtration rate associated with drug administration were not marked. It is suggested that the reduction in urine volume results from a relative depletion of body sodium, the latter

Part B included: No

diminishing the delivery of filtrate to the distal nephron. The increased urine osmolality on the other hand, is apparently due to the specific effect of the drug on the diluting segment within the renal tubule, as well as to the diminution in flow through the distal nephron.

Findings of significance:

The aldosterone antagonist, spirolactone, given in association with hydrochlorothiazide during the antidiuretic phase in patients on a low sodium diet did not alter urine flow or urine osmolality. On the other hand, it did result in an increase in sodium excretion and a fall in potassium excretion. Chloride excretion was not appreciably affected.

Serial No. NHI-235
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Effect of Chlorothiazide on Urinary Dilution and Concentration in the Dog.

Principal Investigator: Jack Orloff

Other Investigators: Laurence Earley
Melvin Kahn

Cooperating Units: None

Man Years -
Total: 9/12
Professional: 9/12
Other: 0

Patient Days: 0

Project Description -

Objectives: Investigations of others have suggested that chlorothiazide interferes with the reabsorption of sodium at diluting sites in the distal nephron. This project was designed to examine further this hypothesis. Since diminishing urinary concentration during the administration of vasopressin and large solute loads may be due to incomplete equilibration of diluted fluid in the distal nephron, it would be expected that an agent which interferes with urinary dilution might exert a measurable effect on urinary concentration under such conditions. Chlorothiazide was therefore given intravenously to dogs undergoing maximal water diuresis in order that the quantitative relationship between increased solute and decreased free water might be assessed. Similarly the drug was given to hydropenic animals receiving vasopressin and large solute loads in order to determine its effect under these conditions.

Progress during the past year: During water diuresis the administration of chlorothiazide resulted in a fall in 'free water' which was quantitatively related to the increased solute excretion produced by the drug. This is consistent with the previous suggestions that these agents interfere with sodium reabsorption at diluting sites in the nephron. When given to hydropenic animals undergoing low rates of solute excretion chlorothiazide did not alter the rate at which concentrated urine was excreted as measured by the calculated loss of solute free water from an isosmotic urine ($Tc H_2O$). As solute excretion is increased in the absence of chlorothiazide $Tc H_2O$ progressively rises to a maximum and then diminishes with further increases in solute

excretion. A possible cause for such a falling TcH_2O is the failure to completely equilibrate the diluted fluid which enters the distal convolution. Equilibration would be impaired by large solute loads which would decrease the transtubular osmotic gradient. Chlorothiazide prevented this fall in TcH_2O suggesting that this agent diminished the degree to which the fluid in the distal nephron was diluted and thereby allowing equilibration to occur more readily. Without changing the rate at which solute-free water is removed by the medullary interstitium, more complete equilibration in the distal convolution would result in a greater TcH_2O.

A complete report of these studies has been submitted for publication.

Major findings of significance: None.

Serial No. NHI-236
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Renotropic effect of chlorothiazide in adrenalectomized rats.

Principal Investigators: Melvin Kahn
Jack Orloff

Other Investigators: None

Cooperating Units: None

Man Years -
Total: 4/12
Professional: 4/12
Other: 0

Patient Days: 0

Project Description -

Objectives: The purpose of this study was to clarify the relationship between the adrenal hormones and the renotropic effects of chlorothiazide. Although indirect evidence suggested that the drug was not a physiologic antagonist of adrenal hormones, a study with adrenalectomized rats suggested a contrary conclusion. An attempt was therefore made to compare quantitatively the acute response to chlorothiazide of adrenalectomized rats and unoperated intact animals.

Progress during the past year: Saline was administered to all rats. Half of the animals in each group, i.e. adrenalectomized or intact, also received 200 mgm/kgm of chlorothiazide by gastric tube. The rats were then immobilized and all urine was collected anaerobically for two consecutive four hour periods.

In the first four hours, the adrenalectomized rats receiving chlorothiazide excreted more sodium, chloride, bicarbonate and water than the adrenalectomized rats which had not received the drug. Similar results were obtained when chlorothiazide was administered to the unoperated animals. Comparison of the chlorothiazide treated animals with their untreated controls, revealed that the increased rate of excretion of chloride, sodium, bicarbonate and water was of the same magnitude in the intact and adrenalectomized animals. During the second four hour period, there was no difference in electrolyte excretion between those animals which had received chlorothiazide and their untreated controls.

Part B included - No

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It is concluded that chlorothiazide may exert a renotropic action in the absence of adrenal hormones. In this circumstance, the effect of the drug is temporally and quantitatively indistinguishable from its effects in intact animals with functioning adrenal glands.

Direction of current research: The above studies are being prepared for publication.

Incidental findings: The rate of ammonium excretion was lower at all levels of urinary pH in the adrenalectomized animals than in the intact animals. This relationship was present in both the treated (with chlorothiazide) and untreated animals.

Serial No. NHI-237
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: ATPase activity of dog renal cortex.

Principal Investigators: Jack Orloff
Maurice B. Burg

Other Investigators: Joseph S. Handler

Cooperating Units: None

Man Years -
Total: 4/12
Professional: 4/12
Other: 0

Project Description -

Objectives: "ATPases" (enzymes which split ATP, giving ADP and inorganic phosphate) are present in many tissues. Recently, it has been shown that "one" of these requires the presence of Na^+ and K^+ and is inhibited by cardiac glycosides. Since this enzyme is active under conditions similar to those favoring cation transport in the intact tissue it has been postulated that the enzyme plays an important, though yet undefined role in cation transport. Other investigators have reported the presence of this particular ATPase in crab nerve membrane, human red cells, and guinea pig kidney. The present studies seek to prepare this enzyme from dog renal cortex, in order to characterize it and investigate the effects of various drugs and hormones on it.

Progress during the past year: The work to date has been concerned with the preparation of the enzyme. Dog renal cortex has been homogenized and differentially centrifuged in a manner similar to that which was successfully employed with crab nerve and guinea pig kidney. ATPase activity has been assayed by measuring release of inorganic phosphate from ATP incubated with the tissue fraction plus Na^+, K^+, Mg^{++}, and a buffer. Since the particular ATPase in which we are interested is inhibited by cardiac glycosides, the amount of inhibition of total ATPase activity by strophanthidin (a cardiac aglycone) has been used as an index for study. In the fractions thus far studied inhibition by strophanthidin has not exceeded 20-30%. This contrasts with reported results elsewhere.

Direction of current research: Future efforts will be aimed at improving the enzyme preparation. Variables being considered include possible species differences as well as alternative methods of isolating and assaying the tissue fractions.

Part B included - No.

Serial No. NHI-238
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Effect of ammonium salts on the sodium and potassium content of kidney slices.

Principal Investigators: Maurice B. Burg
Jack Orloff

Other Investigator: Joseph S. Handler

Cooperating Units: None

Man Years - Patient Days: 0
Total: 4/12
Professional: 4/12
Other: 0

Project Description -

Objectives: The injection of ammonium salts into the renal portal circulation of the chicken kidney interferes with sodium reabsorption and potassium secretion. Furthermore it has also been noted that the potassium concentration of cortical slices is reduced upon incubation in various ammonium salts. The present studies were designed to reinvestigate the latter phenomena in rabbit kidney slices.

Major Findings: In preliminary studies it has been found that incubation of renal cortical slices of rabbits in ammonium chloride in concentrations varying from 2 mM/L to 20, resulted in a diminution in the potassium content of the tissue slices without significant alterations in either the sodium or water content. These phenomena are analogous to those reported in red cells and may indicate that the ammonium ion can substitute for potassium on the carrier.

Proposed course: These studies are being continued in an effort to dissociate the effect of changes in medium pH per se and the ammonium content of the bathing solution on the net changes in the electrolyte composition of cortical slices. The effect of inhibitors of cation transport is also being investigated.

Part B included - No.

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Serial No. NHI-239
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Effect of ammonium salts on electrolyte excretion in the chicken.

Principal Investigator: Jack Orloff

Other Investigators: Melvin Kahn
Luis Brenes

Man Years:
Total: 10/12
Professional: 10/12
Other: 0

Patient Days: 0

Cooperating Units: None

Project Description -

Objectives: In a previous report it was noted that the injection of ammonium chloride into the leg vein of a chicken caused a decrease in the tubular reabsorption of sodium and chloride on the infused side. The present studies were undertaken to reinvestigate these effects as well as to determine the effects of ammonium salts on the excretion of potassium and hydrogen ions.

Progress during the past year: The ipsilateral increase in sodium and chloride excretion following leg vein infusions of ammonium chloride was confirmed. Similar results were obtained with a variety of ammonium salts, including ammonium acetate, nitrate, etc., indicating that the effect is independent of the anion infused. Furthermore it has been established that diuresis is not conditioned by the rate of ammonia excretion, nor by alterations in systemic acid-base balance. It is likely that the inhibitory effects of ammonium salts on electrolyte transport in the kidney are dependent upon the concentration of ammonia within the renal tubular cells and/or in the peritubular areas.

In association with the natriuresis, net secretion of potassium into the tubular urine is diminished by the leg vein injection of ammonium salts. On the other hand net hydrogen ion secretion as estimated by the sum of titratable acid excretion plus ammonia minus bicarbonate excretion when corrected for filtration rate is unaffected by the addition of ammonium salts to the peritubular blood. By analogy with the effects on other cells it has been tentatively concluded that the inhibition of electrolyte transport due to ammonia may be secondary to interference with net potassium uptake by the renal tubule cells.

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Direction of current research:

Attempts to study the same phenomena in the dog by infusion of ammonium chloride directly into the renal artery have been initiated. The results thus far have not been interpretable since there is an associated precipitous drop in glomerular filtration rate on the injected side.

Incidental findings:

No consistent effect on uric acid excretion has been observed following the injection of ammonium salts. Furthermore the infusion of l-alanine, glycine and l-glutamine, amino acids known to increase the rate of ammonia production in other species, did not result in a unilateral increase in ammonia excretion. Instead, there was a slight bilateral augmentation in ammonia excretion.

Part B Included: No

Serial No. NHI-240
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Defect in urinary concentrating ability in some patients with keratoconjunctiva sicca (Sjogren's Syndrome)

Principal Investigator: Melvin Kahn

Other Investigators: Martin Wohl (NIAMD)
Arthur D. Merritt (NIDR)
Kurt Bloch (NIAMD)

Cooperating Units: National Institute of Arthritis & Metabolic Diseases
National Institute of Dental Research

Man Years:
Total: 3/12
Professional: 3/12
Other: 0

Patient Days: 0

Project Description:

Objectives: During the course of a systematic study of patients with Sjogren's Syndrome, it was noted that one of the patients exhibited a depression of urinary concentrating ability. This study was undertaken in order to elucidate the nature of the renal defect.

Progress during the past year: Ten patients have been studied. After eighteen hours of dehydration four patients had a distinct impairment in urinary concentrating ability (360 to 650 mOsm/L); in one this was slightly depressed (790 mOsm/L). All others were able to concentrate normally. Similar results were noted with vasopressin administration.

The patients with the concentrating defect had endogenous creatinine clearances of 66 to 101 ml/min/1.73M^2. PSP clearance was slightly depressed. The urine was free of albumin and sediment. Blood electrolytes, osmolality, urea and creatinine were normal. There was no evidence of endocrine or hematologic abnormality.

The average daily water intake of these patients was 2200 to 3600 ml/day/1.73M^2. Because of the dry mouth, symptomatic of Sjogren's Syndrome, it was thought that perhaps there was an excessive intake of water with a

Part B included: No.

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consequent unresponsiveness to ADH. Water intake was restricted to about 1000 ml/day for 3 to 12 days in three patients. Subsequent dehydration and pitressin administration resulted in no evidence of improved concentrating ability.

The patient with the most severe defect was studied on a restricted salt diet and was able to conserve sodium. When solute excretion was progressively increased under maximal ADH stimulation, urine became dilute at an osmolal clearance of 2 ml/min and free water progressively increased as solute excretion increased. (Urine concentration decreased to 200 mOsm/L). Inulin clearance was 65 to 75 ml/min/1.73M^2.

There is no obvious explanation for the concentration defect at this time. Skin and muscle biopsies of three of these patients have shown a vasculitis of the small vessels. Similar lesions are known to occur in the parenchymal organs in this disease. It is therefore assumed that a vasculitis has involved the kidney and adversely affects the countercurrent mechanisms of the medulla with only mild affect on the glomerular tufts.

Direction of current research: It is planned to test the diluting ability during water loading.

Serial No. NHI-241
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Mechanism of Active Cation Transport in Human Red Blood Cells.

Principal Investigators: Joseph F. Hoffman
Hubert E. Ryan

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.5
Professional: 1.5
Other: 0

Patient Days: 0

Project Description -

Objectives: The primary aim of this work is to establish the molecular basis for the mechanism of active cation transport in human red blood cells. The reports submitted in past years have summarized two aspects of the approach to this problem necessarily basic to the current work. These were the development and characterization of a system of ghosts able to pump Na and the demonstration that the pump utilizes directly high energy phosphate derived from intracellularly produced ATP.

Progress during the past year: Certain additional properties of the ghost system have been studied with the aim of correlating the activity of the Na pump with a specific membrane ATPase. This ATPase was previously shown (by R. L. Post) to be glycoside-sensitive and to require both Na and K for activation in a manner similar to the activation of the active transport system in intact red blood cells. The present report compares the effect of various substrates and agents on the ATPase activity (measured with isolated membranes, permeable to ATP) and active Na transport (measured in reconstituted ghosts) with the following results: both the pump and the ATPase are specific for ATP; neither utilize ADP, ITP, UTP or GTP. Both are inhibited by NaF or $CuCl_2$ but neither are affected by IAA; NaN_3 or Na_3AsO_4. The effect of pH and the action of strophanthidin and dihydrostrophanthidin are the same on both the enzyme and the pump. Mg^{++} is required by both for activity. Ca^{++}, at all concentrations, inhibits both the ATPase and the pump. It is of interest that the effect of Ca^{++} on Na transport is asymmetrical since the inhibition is produced only when Ca^{++} is present

intracellularly; conversely, strophanthidin has been shown to act only from the outside. These and other results provide further evidence that this ATPase represents the pump mechanism or is an intimate component of it.

Direction of current research:

1) isolation (solubilization and purification) of the membrane ATPase and 2) evaluation of the fate of the phosphate split from ATP from the viewpoint of (a) simple hydrolysis or transfer to an intermediate (b) the involvement of lipid, particularly phosphatidic acid (in collaboration with Dr. M. Vaughan).

Part B included - Yes

Part B.

Publications:

Hoffman, J.F., Tosteson, D.C.and Whittam, R. Retention of Potassium by Human Erythrocyte Ghosts. Nature, 185:186, 1960.

Tosteson, D.C. and Hoffman, J.F. Regulation of cell volume by active cation transport in high and low potassium sheep red cells. J. Gen. Physiol., 44:169, 1960.

Serial No. NHI-242
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Mechanism of water movement across membranes.

Principal Investigators: Joseph F. Hoffman
Victor W. Sidel

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.5
Professional: 1.5
Other: 0

Patient Days: 0

Project Description -

Objectives:
Project I: To study the theoretical models for water transfer through membranes by measuring the movement of water and electrolytes through a physical membrane model.
Project II: To apply the deviations from classical theory found in the model to the study of a physiological system.

Progress:
I: A model system, consisting of two aqueous phases separated by a liquid non-aqueous mesityl oxide "membrane" was used to study the flux ratio hypothesis of Ussing. Unidirectional water flow, measured by THO fluxes, and net water flow, measured volumetrically, were determined simultaneously under various gradients imposed by NaCl. Significant differences between activity ratio and flux ratio were observed. For instance, given 0.1 molal NaCl and 5.0 molal NaCl in the opposing compartments, the activity ratio is 1.24 and the measured flux ratio is 1.66. The possibility of droplet movement through the mesityl oxide was eliminated by comparing the flux of Na^{24} with THO flux. The discrepancy between activity and flux ratios in this system does not appear to be due to either active transport or bulk flow. The discrepancy persists in a smaller model in the absence of stirring but disappears, as expected, in classical isopiestic bell jar experiments.

Measurements of the water content of the mesityl oxide in equilibrium with water at various activities were performed using both THO and infra-red spectroscopy. Both methods show a highly significant departure from

linearity between water activity and the water content of the mesityl oxide in equilibrium with it. This non-linearity, assumed to persist at the two interfaces of the model, may explain the unexpectedly high flux ratio observed.

II. Preliminary studies of unidirectional (THO) water flow through the bladder of Bufo Marinus mounted in a Ussing chamber have been performed. When the water activity is raised on the mucosal side of the bladder by substitution of dilute Ringer's solution, the unidirectional water flux appears to increase from mucosa to serosa. However the flux from serosa to mucosa does not appear to decrease, as would be demanded by a bulk flow hypothesis.

Direction of Current Research:

I. Experiments on unidirectional water fluxes in the absence of a gradient remain to be completed, as does the more accurate determination of the water content of mesityl oxide under the various experimental conditions. Attempts are also being made to measure the diffusion of water in mesityl oxide using the capillary method of Anderson.

II. An Ussing chamber designed to permit the simultaneous measurement of net water flow and unidirectional water flow across toad bladder is being set up. These measurements will allow the application of the theory developed in Project I to the interpretation of water movement across this membrane.

Serial No. NHI-243
1. Kidney and Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Cardioglobulin

Principal Investigators: Stephen Hajdu, Edward Leonard

Other Investigators: Wade Jefferson

Man Years:
Total: 2
Professional: 2
Other: 0

Patient Days: None

Project Description -

Objectives: To determine the role of the cardioglobulin system in mammalian plasma. Attempts were made to observe the cardiovascular effects of cardioglobulin depletion in mammals.

Progress during the past year:

A. Depletion of cardioglobulin over prolonged periods. It was thought that this could be accomplished by massive exchange transfusions in which the blood of the experimental animal was replaced by cardioglobulin-free blood, at intervals frequent enough to keep plasma cardioglobulin concentration depressed. The method required an inlying cannula system through which the exchanges could be made without operative intervention each time. This was accomplished in Sprague-Dawley rats by inserting a polyethylene catheter with a curved tip into the left carotid artery and then into the descending aorta. A piece of nylon monofilament fishing line was used as an obturator, and with appropriate capping of the end of the catheter system, which emerged through a skin opening just posterior to the left eye it was possible to allow the animal to run free during the intervals between exchanges. Cardioglobulin was depleted by rapidly removing about half of the blood volume and replacing with a mixture of rat red blood cells and human fraction V in Krebs solution. This was done 6 times over a twenty minute period, and three hours later the whole procedure was repeated in order to wash out any protein that might have shifted from interstitial to vascular space following the first exchange.

It became apparent that some rats could survive daily exchanges of this sort for three to five days without any obvious ill effects. At autopsy

Part B included: Yes

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a fifty per cent increase in the weight of the liver and a two to four-fold increase in the weight of the spleen was found. Measurements of cardioglobulin C concentration showed normal values 24 hours after exchange. In protocols set up to determine the rapidity of cardioglobulin C recovery after depletion, it was found that the concentration was back to 50% of normal by 3 hours and approximately normal by 6 hours following exchange. Electrophoretic fractions (as determined by paper electrophoresis) showed the same pattern of recovery, except for the gamma globulins which lagged behind the others.

If splenectomy was performed just before the exchange transfusion, recovery of cardioglobulin was greatly slowed. The effect of splenectomy on synthesis rates of other proteins was not examined.

These experiments made it clear that it was not practical to attain prolonged depletion of cardioglobulin by exchange transfusions in a normal rat, because of the apparent capacity for rapid synthesis of new protein. The possibility of exchanges in animals with experimental liver injury has been entertained.

B. Depletion of cardioglobulin in acute experiments. The capacity for rapid turnover of cardioglobulin in the rat, and the depletion of the cardioglobulin system when added to an isolated frog heart suggested that perhaps utilization of cardioglobulin by the rat heart might occur rapidly and that depletion of the system would be reflected in changes of myocardial contractility that could be observed in acute experiments.

A rat "heart-lung'brain" preparation was worked out for these experiments, in which all tissues below the diaphragm were excluded from the circulation. Blood from the aorta traversed parallel circuits - one perfusing the upper part of the animal, the other going through an external circuit consisting of an adjustable peripheral resistance, venous reservoir, and cannula to the inferior vena cava. Blood pressure, heart rate, shunt flow and venous pressure could be monitored. The plan was to compare cardiac performance in normal rat blood with cardioglobulin-free blood.

Despite some early experiments to the contrary, it would now appear that cardiac performance in this preparation over a period of 2-4 hours is not influenced by the cardioglobulin system. However more experience is needed before this conclusion can be stated with certainty, since a number of experimental difficulties may occur some of which have been appreciated only recently. For example, replacement of cardioglobulin-free blood with normal blood has been difficult because of the problems of adequate mixing in the animal and because of rapid destruction of cardioglobulin (see below). These problems have been solved by using healthy donor animals in cross-circulation with the experimental preparation when the time comes to replace the cardioglobulin, but our experience with this approach has been limited.

C. Destruction of cardioglobulin. In relation to some of the experimental difficulties noted above, it was found that mere circulation of rat blood through an external shunt resulted in destruction of cardioglobulin C.

For example, in a normal rat with a shunt from carotid artery through peripheral resistance to venous reservoir and back to jugular vein Cardioglobulin C concentration dropped to extremely low values within 2-3 hours.

This led to the testing of the effect of various foreign surfaces on cardioglobulin stability. The results suggested that despite small differences among various materials (polyethylene, polyvinyl, stainless steel, glass), the more important variables had to do with the air-blood interface which was large in the original hsunt system because of the blood filter. (It should be added that certain materials - a commercial grade nylon tubing and natural rubber - appeared to cause much more cardioglobulin destruction than did the other substances tested). We have subsequently found that no detectable destruction of cardioglobulin C occurs in a shunt without filter and with minimal air-blood interface (materials in shunt being polyethylene, stainless steel, and polypropylene).

The threat of cardioglobulin C destruction in rat blood is always close at hand, since merely centrifugation at 5000 rpm in a Serval is sufficient to cause liberation of a destroying substance from one of the formed elements. It is interesting that this material comes not from platelets or white cells, but from the erythrocyte, and is probably one of the proteolytic enzymes of the red cell membrane described by Neurath. Fractionation of ghosts according to Neurath's procedure has yielded a non-dialyzable material that causes loss of cardioglobulin C activity on incubation.

Direction of current research:

Now that certain of the experimental difficulties with the "heart-lung-brain" preparation have been eliminated, a more refined attempt will be made to determine whether alterations in cardioglobulin concentrations have any effects on the myocardium in acute experiments. If not, a further effort will be made to deplete experimental animals in chronic experiments, perhaps by exchange transfusion in combination with controlled liver injury or possibly by injection of destroying enzyme such as the material described by Neurath.

Part B.

Publications:

(1) Leonard, E. and Hajdu, S. Cardioglobulin. Clin. Res. 8, 187, 1960.

(2) Leonard,E. and Hajdu, S. The action of electrolytes and drugs on cardiac muscle. Handbook of physiology. Amer. Physiol. Soc. In press.

Serial No. NHI-244
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Measurement of Renal Clearance of C-14 Carboxyl-labeled Inulin in Humans

Principal Investigator: Ernest Cotlove

Other Investigators: Dr. Louis V. Avioli, Radiation Branch, National Cancer Institute

Man Years:
Total: 4/12
Professional: 4/12
Other:

Patient Days: None

Project Description -

Objectives: Carbon-14 carboxyl-labeled inulin has been shown to be a reliable tracer for inulin in previous studies in this laboratory, on the basis of standard clearance measurements in rats, dogs, and humans. Radioassay of C-14 has been performed by an infinite thickness liquid counting method developed in this laboratory. This method, though simple and accurate, is limited in sensitivity, so that about 50 microcuries are required for a two-hour renal clearance procedure in an adult man. A more sensitive method of radioassay of C-14 inulin in plasma would be desirable, particularly for studies involving prolonged or repeated administration of C-14 inulin. Accurate radioassay of C-14 inulin is of interest since it can be of unique value in studies requiring analysis of inulin at trace concentrations, or in the presence of chemically similar substances.

Progress during the past year:

The infinite thickness liquid counting method, using a 7-inch diameter gas flow detection chamber adapted in this laboratory for the use of large samples (15 ml. of blood or urine), has a lower limit of sensitivity for C-14 of about 2 microcuries per liter. Attempts were made to measure lower concentrations by dry plating of urine, plasma, or plasma filtrates, or by conversion to carbon dioxide and barium carbonate, but consistent results could not be obtained. It was not possible to isolate inulin quantitatively prior to plating or to counting by liquid scintillation. However, it has been found practical to utilize the sensitivity of liquid scintillation to

Part B included: No.

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to count aqueous samples of diluted urine and of protein-free supernatant and of plasma, using a modification of a scintillation medium and procedure developed by Dr. George Bray in this laboratory. Reproducible counting could be achieved in plasma supernatants by zinc hydroxide precipitation at only 2-1/2 fold dilution, without loss of the trace amount of inulin in the precipitation step. The lower limit of measurement could thus be reduced to about 0.5 microcuries per liter. However, quenching of counting occurred amounting to 5 to 15% in plasma supernatants, and 10 to 35% in different urines. Almost all the quenching material in urine, mainly colored compounds, could be removed with activated charcoal, but this also adsorbed most of the C-14 inulin. The degree of quenching, although varying from sample to sample, was found to be consistent in replicates of the same sample. It was therefore possible to correct for quenching in each sample with internal standards, by adding to duplicates of each unknown an exact amount of a standard C-14 solution with hundred-fold or greater concentration of radioactivity. The unknowns were corrected by a factor obtained from the degree of reduction in counting rate of the added standard. Preliminary results with this procedure compared favorably with the infinite thickness liquid counting method, which previously was shown to have an accuracy within $\pm$ 1%.

The specific activity of the C-14 inulin employed in these studies is 1.4 microcuries per milligram, so that the plasma concentration of inulin at the lower limit of sensitivity of the liquid scintillation method described is about 0.04 mg%, and is about 0.15 mg% at the lower limit of the infinite thickness liquid counting method. Three separate measurements of C-14 inulin clearance within several weeks in a 55 kg. female patient gave average values of 124 ml/min. in one experiment at a plasma inulin concentration about 0.20 mg%, and 127 and 131 ml/min. in two experiments at a concentration about 0.05 mg%. These results are consistent with previous evidence obtained in this laboratory showing that the renal clearance of inulin is unaffected by plasma concentration down to trace concentrations.

Direction of current research:

The measurement of C-14 inulin clearance, as an index of glomerular filtration rate, is being conducted with Dr. Avioli as part of his wider study of the effects of therapeutic X-ray radiation on specific renal functions in humans. Further comparisons will be made between the two counting methods, and between the clearance of radioactivity and of chemically analyzed carrier inulin. It is planned to collect bile with a duodenal tube when feasible during infusion of C-14 inulin, in order to detect possible biliary excretion of radioactivity, which has been shown to occur in the rat to a small extent.

Serial No. NHI-245
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Myocardial chloride content in relation to experimental cardiac failure.

Principal Investigator: Ernest Cotlove

Other Investigators: Nicholas A. Yankopoulos, James O. Davis, and Mary Trapasso.

Man Years:
Total: 8/12
Professional: 8/12
Other:

Patient Days: None

Project Description:

The alterations in the myocardium accompanying cardiac failure and their relationships to the functional deficiencies have been of continuing interest. The interpretation of reported changes in the water and electrolyte composition of failing myocardium depends upon accurate analysis of total tissue chloride, evaluation of chloride distribution, and appropriate experimental conditions enabling assessment of certain relevant factors.

Progress during the past year:

A simple method for chloride analysis of myocardium was adopted, involving coulometric-amperometric titration of a water extract of dried, fat-free tissue. This method was validated by an isotope dilution procedure using radioactive Cl-36, and the preparative steps of drying at 105°C. and of ether extraction of fat, were each shown to cause no loss of chloride. The total chloride of muscle could be measured within $\pm$ 2% accuracy. Another source of error within this limit is introduced by the chloride of red cells remaining in the analyzed tissue. The independent estimation of extracellular volume of dog heart by equilibration of exogenous extracellular indicators was not feasible, but a separate study was conducted to evaluate the distribution of radioactive inulin in rat skeletal muscle under conditions approaching equilibrium, as described in an accompanying report.

The chloride, sodium, potassium and water contents of myocardium were measured in normal dog hearts and in three groups with experimental

Part B included: Yes.

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cardiovascular lesions. The chloride content of myocardium per 100 grams of fat-free solids increased 22 to 60% in the experimental groups, accompanied by parallel increases of sodium. The ratios of the increments in myocardial sodium to chloride were essentially the same as the sodium/chloride concentration ratio of 1.22 in extracellular fluid, averaging 1.18 in dogs with combined pulmonic stenosis and tricuspid insufficiency resulting in chronic congestive failure, and 1.19 in dogs with tricuspid insufficiency; a lower ratio of 0.8 in the third group with thoracic inferior vena cave constriction was not significantly different since the smaller increments in this group involved a larger error in the ratio. Increases in total myocardial water were found in the experimental groups and corresponded in magnitude to addition of isotonic sodium chloride. On the other hand there were no significant changes in myocardial potassium per 100 grams of fat-free solids, or in calculated intracellular water.

These results, taken together, are consistent with the view that the increases in total myocardial water, sodium and chloride in the dogs with experimental lesions represented extracellular fluid and can be described as myocardial edema. On the basis of considerable evidence in skeletal muscle, it is unlikely that cellular sodium would have increased without reduction in cellular potassium. The extracellular ratio of the increments in sodium and chloride also makes it unlikely that a shift of chloride to the cellular compartment occurred in the experimental groups. Essentially all the myocardial chloride, as well as the increment in chloride, is probably also extracellular, as has been strongly indicated in rat skeletal muscle on the basis of earlier studies in this laboratory, which have been further supported by recent preliminary experiments using improved methods. The effects of the experimental lesions in producing elevated right atrial pressure, hypoproteinemia, and hyperaldosteronemia also would be more likely to produce an increase in extracellular rather than intracellular fluid. Comparison of these three effects, and also of cardiac hypertrophy and duration of cardiac failure, with the degree of alteration in water and electrolyte composition in the three experimental groups and a fourth group with pulmonic stenosis and cardiac failure suggested that the major pathogenic factor in myocardial edema formation was chronic passive congestion of the myocardium due to elevated right atrial pressure. It would appear that the altered myocardial water and electrolytes in cardiac failure do not constitute the primary defect, although these may contribute to further depression in myocardial function.

Incidental findings of significance:

The adrenal cortex of the dogs in this study was also analyzed for water and electrolyte composition. The sodium and chloride contents showed greater variability than those of myocardium, and the somewhat low ratio of total tissue sodium to chloride presents the possibility of some intracellular chloride in the adrenal cortex. Hypertrophy of the adrenal cortex occurred in the experimental groups, particularly in the dogs with caval constriction. The water content of the adrenal cortex was increased in the dogs with marked chronic passive congestion, and the increment in chloride though small suggests the presence of extracellular edema.

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Direction of current research:

This project has been completed.

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Part B.

Publications.

(1) N. A. Yankopoulos, J. O. Davis, E. Cotlove, and M. Trapasso: "Studies of the Mechanism of Myocardial Edema in Dogs with Chronic Congestive Heart Failure," American Journal of Physiology, 199:603, 1960.

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Serial No. NHI-246
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Chloride and potassium equilibria across the cell membrane

Principal Investigator: Ernest Cotlove

Other Investigators: None

Man Years:
Total:3/12
Professional: 3/12
Others:

Patient Days: None

Project Description -

Objectives: A satisfactory theory of cell functioning must account for the known facts of its distinctive ionic composition and of the alterations occurring in response to disturbances in the extracellular environment, and will need to include more complete information on these aspects. Although the predominant anion in extracellular fluid is chloride, its concentration in most body cells is in doubt, as are the factors which can affect its distribution across the cell membranes. Various assumptions regarding the distribution of chloride in tissues have been extensively employed as the basis of reference for estimating the distribution and cellular concentration of many other substances analyzed in tissues. The problems which have made the assessment of chloride partition in tissues uncertain are now considerably nearer solution. Previous work in this laboratory has defined the factors involved in the independent measurement of extracellular volume with indicators such as saccharides, has shown C-14 inulin to be a dependable tracer of inulin, has developed an accurate method of radioassay of C-14 inulin in tissues by infinite thickness liquid counting, and has developed simple methods of chloride analysis in tissues which were validated by an isotope dilution method using radioactive Cl-36.

Progress during the past year:

It has been shown by previous studies in this laboratory that saccharides of the size of sucrose or larger, employed as extracellular indicators because they do not appear to penetrate cell membranes, also are slow to reach equilibrium distribution in less vascularized areas of the extracellular compartment. The required period of equilibration is of the

Part B included: No.

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order of 24 hours. The present experiments are conducted on nephrectomized rats, which develop elevated plasma potassium concentration and acidosis. These effects should provide a test of major hypotheses of chloride distributior one advanced by Conway relating the cell chloride concentration to the plasma potassium concentration; and another suggesting that extracellular acidosis produces a chloride shift not only into red cells but also into body cells presumably including muscle. Both hypotheses predict an increase in cell chloride under these conditions which would exaggerate any difference between the measured volumes of distribution in tissue of naturally present chloride and of injected C-14 inulin so as to be readily detectable with the methods developed. An additional practical advantage of the use of nephrectomized rats is to reduce the amount of C-14 inulin required for each experiment to 10 microcuries compared with 300 microcuries required for a 24 hour infusion in an intact animal (which excretes concurrently about 98% of the infused dose).

Experiments were performed to establish the accuracy of chloride titration and of radioassay of C-14 inulin in tissues with the preparative procedure developed to allow analysis at low concentrations. Known amounts of C-14 inulin added to rat skeletal muscle or liver in vitro were quantitatively recovered within ±2%. Isotope dilution measurement of naturally present chloride confirmed the accuracy of titration within ±2%.

Bilateral nephrectomy was conducted under ether anesthesia through an anterior abdominal approach. Successful experiments have thus far been completed in two rats, which remained in apparently good condition for 26-28 hours after nephrectomy and showed no infection at sacrifice. The period of equilibration after intravenous injection of 10 microcuries of C-14 inulin was 24 to 25 hours. An indication of the reliability of analysis is given in one rat by comparison of analyses of symmetrical muscles removed from opposite legs: chloride space of 11.1% on right, 11.1% on left; and C-14 inulin space of 10.5% on right, 10.3% on left. In the second rat the chloride space in muscle was 10.8% and C-14 inulin space was 10.3%. The ratio of inulin to chloride space of 0.94 and 0.96 in the two rats compares with the average ratio of 0.93 obtained in a previous study in this laboratory on intact rats given 15 hour infusions of non-radioactive inulin, based on less adequate analytical methods. The near equality of inulin and chloride spaces in skeletal muscle of the nephrectomized rats does not substantiate a chloride shift into muscle cells expected on the basis of the foregoing hypotheses. From the magnitude of the effect predicted by the Conway hypothesis, with the plasma potassium of about 7 mEq./L. in the nephrectomized rats, the calculated inulin to chloride space ratio would be about 0.75, in contrast to the ratio of about 0.95 in these preliminary experiments. It would appear that the simplified view of chloride distribution across the cell membrane as occurring passively in accordance with the potential gradient determined by the potassium concentration ratio may not describe the behavior of mammalian muscle in vivo, although it appears to explain results on amphibian muscle in vitro under certain conditions.

007

In the rat heart the C-14 inulin space was 0.91 and 0.95 of the chloride space; corrected for red cell chloride these ratios would be a few per cent higher. In the liver the C-14 inulin of 77% and 65% were near the usual total water content of about 70%, and several times greater than the corresponding chloride spaces of 21% and 24%. These results are in accord with those of others showing biliary excretion of inulin in the rat. Hepatic uptake and excretion of inulin may be the main or only site of loss of inulin from the extracellular space. The total body dilution of radioactivity, calculated as the ratio of the amount injected to the terminal plasma concentration, was 38% and 40% of body weight in the two rats, considerably higher than a reasonable figure for extracellular volume. The high value is not due to metabolic splitting off of a small C-14 labeled molecule, since chemical measurement of the alkali-stable fraction of the C-14 inulin in plasma and injected solution gave the same figures for total body inulin space.

Incidental findings of significance: None.

Direction of current research:

Further experiments will be conducted to determine the consistency of these findings and their relation to plasma potassium level and degree of acidosis, to analyze other organs for comparison with muscle and liver for detection of uptake by macrophages or other cells, and to determine the loss of radioactive inulin by biliary excretion in relation to the increase in volume of dilution.

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Serial No. NHI-247
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Validation of chloride analysis in biological fluids.

Principal Investigator: Ernest Cotlove

Other Investigators: None

Man Years:
Total: 2/12
Professional: 2/12
Other:

Patient Days: None

Project Description -

Objectives: The measurement of chloride in biological fluids, although not subject to the very wide discrepancies in results obtained with different methods of tissue chloride analysis, has involved continuing questions. These questions have centered in several areas: the accuracy of analysis in the presence of protein or of high fat content due to possible chloride binding; possible interference introduced by high organic content as in concentrated urine or bile; and the variability of results on urine, particularly after storage.

Progress during the past year:

A reliable reference method of chloride analysis, developed in this laboratory, has been applied to these problems. This reference method, which is exacting and time-consuming, employs isotope dilution techniques. Radioactive Cl-36 is added to the biological fluid sample; radioassay, by an infinite thickness liquid counting method developed in this laboratory, is performed after digestion of the solution with hot dilute sodium hydroxide; and chloride is then isolated in successive stages of purification to constant specific activity. Radioassay and titration are performed at each of the three stages: dry ashing; oxidation, diffusion as chlorine gas, and reduction; and distillation of HCl. The same samples were also analyzed directly by the simple, rapid procedure of automatic coulometric-amperometric titration developed in this laboratory.

Six human sera were analyzed by automatic titration of the whole serum and by isotope dilution. Four of the sera contained normal amounts of lipid; two appeared milky and contained 1.5 and 2.4 grams% of lipid. The chloride

Part B included: Yes

results by automatic titration averaged 99.9% of the isotope dilution results; the individual comparisons were 99.0%, 100.2%, 100.5%, 101.0% and in the two high-lipid sera were 100.4% and 98.0%, respectively. Similar comparisons on two samples of dog pancreatic secretion, which were viscid and milky, gave titration results equal to 99.3% and 100.2% of those of the reference method. The accuracy of direct titration of a sample of human urine containing 12 gms./L. of protein was 100.5%, and of a highly concentrated sample of cat urine containing 435 mEq./L. of chloride was 101%.

A careful comparison was made of results of automatic titration on whole serum and on protein-free supernatants of serum precipitated with neutral or with acid zinc sulfate followed by sodium hydroxide, using high dilution to minimize volume effects due to precipitated solids, and standards precipitated under identical conditions as controls. The results following protein removal averaged 99.8% of the titration results on whole serum, with a range of 99.3 to 100.3% in five sera, a difference which was not significant.

Direct titration of samples of concentrated gall bladder bile samples from dogs, containing less than 1 mEq./L of chloride, showed interference from bile salts. The variation in results of $\pm$ 20%, was reduced to half by increasing the acidity of the titration medium from the usual 0.1 N to 0.3N nitric acid. Addition of chloride to the bile to yield concentrations as low as 5 to 10 mEq./L., reduced the error of titration to less than 3%, as shown by recovery of the added chloride to the almost chloride-free samples. No difficulties were encountered in direct titration of fresh gastrointestinal fluid samples from stomach, small, or large intestine of dogs, or of gastric fluid samples from humans.

Urine samples, titrated after a day or more of storage, showed higher chloride figures than obtained initially on the day of collection. This finding, which has been reported by others using different methods, also occurred with other biological fluids to varying degrees. The increases, amounting to 5 to 20% or more, were found to be related to contamination of the samples with resultant formation of sulfhydryl groups which combine with silver ion titrant. The sulfhydryl interference could be removed by a simple oxidation procedure with alkaline perborate prior to titration, which then yielded the initial, correct value for chloride.

Incidental findings of significance:
None.

Direction of current research:
This project has been completed.

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Part B.

Publications.

(1) Cotlove, E.: "Chloride", in Seligson, D., ed., Standard Methods of Clinical Chemistry, Vol. III, Academic Press, New York (in press).

Serial No. NHI-248
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Automatic titration with direct read-out of chloride concentration

Principal Investigator: Ernest Cotlove

Other Investigators: Hiroshi H. Nishi, Chemist, Clinical Chemistry Service, Clinical Pathology Department, Clinical Center

Man Years:
Total: 3/12
Professional: 3/12
Other:

Patient Days: None

Project Description -

Objectives: The analysis of chloride in biological fluids such as serum, spinal fluid, sweat, gastrointestinal fluid, or urine is of value in experimental and clinical evaluation of disorders of electrolyte metabolism. A method and instrument for chloride analysis by automatic coulometric-amperometric titration were developed in this laboratory and are being employed increasingly widely in both clinical and research laboratories. The routine clinical analysis of chloride could be further speeded and simplified by provision for indication of the result directly in units of concentration.

Progress during the past year:

An auxiliary to the automatic titrator has been designed which provides direct indication of chloride concentration by performing two functions. An adjustable time delay control, set at the measured blank titration time, automatically subtracts the reagent blank time before starting the auxiliary's timer, thus yielding net titration time. The net titration time appears in units of mEq./L. on the digital register of the auxiliary's timer, which was selected for the appropriate speed, once the coulometric current has been adjusted with the auxiliary's rheostat to give correct readings of a chloride standard.

The direct read-out auxiliary, connected to the automatic titrator, has been in regular use in the clinical chemistry laboratory for almost a year,

Part B included: Yes.

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and has proved to be simple to operate and consistently accurate. The results obtained by twelve medical technologists during a period of 50 consecutive days were evaluated. An autopipet was used to measure 0.1 ml. samples of various biological fluids. The coefficient of variation of daily averages of triplicate measurements of the chloride standard was ± 0.4%, and of single daily titration of pooled sera was ± 0.7% or less. The coefficient of variation of duplicate titrations of biological fluids was ± 0.5%. Daily setting of the "blank time delay" control rendered negligible the variability due to this correction.

Comparison of direct read-out measurements of concentration with those obtained simultaneously from readings of the seconds timer of the titrator followed by calculation showed identical results on the average, and a mean difference in individual titrations of ± 0.5%.

Incidental findings of significance: None

Direction of current research:

This project has been completed, and the instrument has continued in regular use in the Clinical Chemistry Service.

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Part B.

Publications.

(1) Cotlove, E. and H.H. Nishi: "Automatic Titration with Direct Read-Out of Chloride Concentration," Clinical Chemistry, in press.

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Serial No. NHI-249
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Renal clearances in the rat by a constant infusion method

Principal Investigator: Ernest Cotlove

Other Investigators: None

Man Years:
Total: 1/12
Professional: 1/12
Other:

Patient Days: None

Project Description -

Objectives: The albino rat is employed extensively as an experimental animal in studies involving measurement of the renal clearance or excretion of exogenous or endogenous substances, or involving knowledge of basic parameters of renal function. However, the measurements reported in the literature of glomerular filtration rate and renal plasma flow vary considerably, due to the inadequacy of renal clearance methods previously available for use in small animals. The standard techniques for renal clearance measurement used successfully in larger animals require stable plasma levels of the test substance over adequate periods of time, accurately timed collection of all urine formed, and sampling of blood for analysis without altering the plasma level during the period of measurement. Constant infusion of exogenous test substances is an essential requirement, but previous techniques to accomplish this in the rat have required anesthesia, operation, and ligation and cannulation of major vessels, with resultant disturbance of normal renal function.

Progress during the past year:

A method for constant intravenous infusion of the unoperated, unanesthetized rat, previously developed in this laboratory, has been simplified to enable its use by persons with ordinary skill. Adequate dilation and prominence of the tail veins are achieved by warming the rat, confined in a holder, on a heated platform. A specified procedure is followed for insertion of a hubless needle attached to fine polyethylene tubing in a lateral tail vein. Once the infusions are properly started they can be continued with few failures for hours or days, and can remain unattended for

Part B included: No.

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periods as long as overnight. During constant infusion of inulin or creatinine stable plasma levels were maintained for periods up to several hours (coefficient of variation of 5 to 7%), as measured by blood sampling from a hind foot vein initially, and subsequently from the foot vein or from the abdominal aorta under light ether anesthesia. In 220 to 350 gram rats, with circulating blood volumes in the range of 10 to 20 ml., the slow removal of 0.3 to 0.5 ml. of blood from a hind foot vein did not cause important change in the glomerular filtration rate. The plasma concentration of constantly infused inulin or creatinine increased less than a few percent in second blood samples taken 5 to 180 minutes after the first.

A simple procedure of urine collection by voiding, spontaneous or induced by tactile or electrical stimulation, was compared as to reliability with a procedure of bladder catheterization. With either procedure the excretion rate of inulin was essentially the same as the constant infusion rate for the average of 34 to 44 periods. The coefficient of variation of individual periods of urine collection by voiding was 11%, and by catheter was 7%. In periods with urine volumes below 2 ml. because of shorter intervals or slower urine flows the variation was not changed with the catheterization procedure but was increased with the voiding procedure.

The inulin clearance in albino rats, as an index of glomerular filtration rate, has been repeatedly measured with the constant infusion method, in the course of other studies. The average inulin clearance of repeated series has been between 0.8 and 1.0 ml/min. per 100 grams body weight, unrelated to strain (Sprague-Dawley or Osborne-Mendel), sex, or recent intake of food. In contrast, other reported studies using a single subcutaneous or intraperitoneal injection of inulin have obtained inulin clearance figures between 0.3 and 0.7 ml/min. per 100 grams. A positive relation of inulin clearance to urine flow appeared in some reported studies and not in others. In this laboratory, although this aspect was not examined separately, no consistent relation appears in the accumulated data.

The constant infusion procedure when properly performed does not stimulate excessive secretion of antidiuretic hormone. A water diuresis can be achieved by infusion of 2.5% dextrose, resulting in urine osmolarity below 100 mosM/L.

Incidental findings of significance: None.

Direction of current research:

In previous studies the relation of inulin clearance to urine flow has been examined in separate groups of rats, which greatly increases the variability of the results. With the constant infusion method, however, it is possible to alter urine flow while maintaining a stable plasma level during each period of collection in the course of the same experiment in individual rats. Such experiments have been initiated and should provide a more critical test of the relationship.

The renal clearance of para-aminohippuric acid at low and high plasma levels, as measures of effective renal plasma flow and of functional renal tubular mass, will be examined with the constant infusion method, since previous reported studies have shown marked discrepancies in these measurements in the rat.

A portion of this project, concerned with description of the method of measurement of renal clearances in the rat by constant infusion, has been completed and submitted for publication.

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Serial No. NHI-250
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Kinetics of renal excretion of glomerular clearance substances

Principal Investigator: Ernest Cotlove

Other Investigators: None

Man Years:
Total: 1/12
Professional: 1/12
Other:

Patient Days: None

Project Description -

Objectives: The measurement of the rate of urinary excretion of a substance following a single intravenous injection is involved in many types of studies, including clinical evaluation of renal function in man, pharmacology of drugs, and investigation of transient states in experimental studies of renal function. Previous studies in this laboratory have shown inulin to be heterogeneous, consisting of smaller alkali-labile, and larger alkali-stable, polyfructosides. The renal clearances of these two fractions, however, were found to be the same when measured under steady-state conditions. Examination of the kinetics of urinary excretion after a single injection of these and other glomerular clearance substances of smaller molecular size would provide information on possible transient-state differences, and indication of extra-renal factors affecting excretion rate under these conditions.

Progress during the past year:

The kinetics of excretion were examined in successive short periods of time after a single injection of C-14 carboxyl-labeled inulin together with non-radioactive carrier inulin. The injection was preceded and followed by a rapid rate of infusion of half-isotonic saline to maintain high urine flow and allow urine collection at intervals of 3 to 6 minutes. The injected solution and urine samples were analyzed for radioactivity by an infinite thickness liquid counting method developed in this laboratory, and for alkali-stable inulin by diphenylamine reaction after heating in dilute sodium hydroxide. Preliminary experiments have shown a definite pattern in the time-course of the ratio of radioactivity to alkali-stable inulin content of the

Part B included: No

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urine. Since the radioactivity comprises alkali-labile as well as alkali-stable molecules, this ratio will be referred to as "apparent specific activity". Compared with the apparent specific activity ratio in the injected solution, there was a decrease of 10% or more in urine samples collected during the first ten minutes after injection, followed by a rise during the next sixty minutes to 10% or more above, and a subsequent return toward, the ratio in the injected solution.

The urine and injected solution were also examined for qualitative differences in polysaccharide fractions by paper chromatography followed by radioautography of the chromatograms. The C-14 inulin, like the non-radioactive carrier inulin, consisted mainly of larger components remaining at the origin in the chromatogram, and lesser amounts of a spectrum of smaller components less mobile than trisaccharides. The injected and excreted radioactivity showed no qualitative differences in chromatographic pattern; with this technique, quantitative differences of the degree noted above would not be detectable.

In the early time-course following a single injection, differential rates of excretion of alkali-labile and alkali-stable inulin fraction can be shown. If the renal clearance of the smaller alkali-labile molecules were less due to partial reabsorption, this could only account for the low ratio of radioactivity to alkali-stable inulin in the earliest urine samples. Subsequently, a progressively decreasing ratio would be expected in contrast to the distinctly rising ratio found. Furthermore, during constant infusion, renal clearances of these smaller and larger molecular constituents of inulin were found to be equal in previous studies in this laboratory. An interpretation consistent with the findings assumes equality in the renal clearance of the two inulin fractions, and inequality in the extra-renal plasma clearance through body capillaries generally. The extra-renal plasma clearance is of considerable importance in the transient state after a single injection. Compared with the larger alkali-stable inulin molecules, the extra-renal plasma clearance of the alkali-labile molecules would appear to be substantially faster. The radioactive inulin, comprising both alkali-labile and alkali-stable fractions, would show a lower plasma concentration and lesser renal excretion immediately after injection than the alkali-stable carrier inulin, followed by a greater renal excretion from the larger extravascular pool, and finally the excretion rates of the smaller and larger molecules would tend to coincide.

Incidental findings of significance:

The "apparent specific activity", measured as described above, of the early urine samples after a single intravenous injection of C-14 inulin in the rat provides a very sensitive measure of the physiological heterogeneity of the radioactive molecules. It also would detect the presence of radioactive molecules with renal clearance markedly different from the carrier inulin. Since this procedure is also simple and inexpensive in its requirement of C-14 inulin (only one microcurie per experiment), it would be useful for preliminary biological screening of C-14 inulin for human use.

Direction of current research:

Some further studies are planned to test the interpretation of the findings and to evaluate the relative influence of extra-renal and renal clearance by similar comparisons with the smaller glomerular clearance substances, sucrose and mannitol. The C-14 radioactive isotopes will be required to allow accurate analysis in the presence of non-radioactive inulin.

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Serial No. NHI-251

1. Kidney & Electrolyte Metabolism
2. Experimental Cardiovascular Disease
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Physiology of Congestive Heart Failure

Principal Investigator: James O. Davis

Other Investigators: Carlos R. Ayers
Charles C.J. Carpenter

Man Years:
Total: 3
Professional: 3
Other: 0

Patient Days: 0

Project Description:

Objectives:

Area I. To determine the mechanisms regulating the secretion and metabolism of aldosterone in secondary hyperaldosteronism.

Area II. To define the biochemical defect in the failing myocardium.

Progress During the Year:

Area I. To determine the mechanisms regulating the secretion and metabolism of aldosterone in secondary hyperaldosteronism.

Project I

1a. Title: Relation of anterior pituitary function to the secretion of aldosterone and corticosterone in conscious dogs.

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1b. Investigators: James O. Davis, Charles C.J. Carpenter, Carlos R. Ayers, and Robert C. Bahn.
1c. Progress During the Year: Project has been completed and paper published. All data were presented in the last annual report.

Project II

2a. Title: Concerning the role of arterial baroreceptors in the control of aldosterone secretion.
2b. Investigators: Charles C.J. Carpenter, James O. Davis and Carlos R. Ayers.
2c. Progress During the Year: The project is complete and paper is written.

Experiments were designed to test the possibility that arterial baroreceptors play a primary role in the regulation of aldosterone secretion. Dogs with hyperaldosteronism and virtually complete Na retention secondary to thoracic inferior vena cava (IVC) constriction were subjected to: 1) chronic denervation of the cervical carotid arterial system including the thyrocarotid arterial junction (5 dogs), 2) chronic denervation of the aortic arch and left cervical vagotomy (7 dogs), and 3) combined denervation of the cervical carotid arterial tree and the aortic arch, bilateral cervical vagotomy and bilateral splanchnic nerve section (5 dogs). Hypersecretion of aldosterone and marked Na retention continued following denervation in all except one dog in which a transient increase in Na excretion occurred. Acute proximal constriction of the common carotid artery failed to produce a significant change in the rate of aldosterone secretion (7 dogs). Acute proximal constriction of the celiac axis and the superior mesenteric artery had no effect on aldosterone secretion rate (2 dogs). These experiments indicate that 1) baroreceptors in the aortic arch and the carotid arterial tree are not essential to the hypersecretion of aldosterone and almost complete Na retention observed in the dog with chronic thoracic inferior vena cava constriction, and that 2) marked reductions in pulse pressure in the carotid arterial system and in the mesenteric arterial tree fail to increase the rate of aldosterone secretion.

Project III

3a. Title: Effects of midbrain transection and subsequent bleeding on aldosterone and corticosterone secretion.
3b. Investigators: James O. Davis, Evelyn Anderson, Charles C.J. Carpenter, Carlos R. Ayers, Webb Haymaker and William T. Spence.
3c. Progress During the Year: The project is complete and paper has been accepted for publication.

Following midbrain transection of 6 normal dogs, secretion rates for aldosterone, corticosterone and Porter-Silber chromogens were not significantly different from values obtained in unoperated dogs; subsequent bleeding increased aldosterone secretion while corticosterone output was unaltered. In 5 dogs with thoracic caval constriction in which there was a high secretion of aldosterone, midbrain transection was followed by a

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reduction in inferior vena caval pressure to a level which was apparently too low to sustain the mechanisms resulting in hyperaldosteronism. In one animal aldosterone and corticosterone output was extremely low despite a high venous pressure. In the 2 dogs with caval constriction in which the venous pressure remained elevated after midbrain transection, the high rate of aldosterone secretion persisted. Bleeding of dogs with caval constriction and midbrain transection resulted in a striking increase in aldosterone secretion. In dogs with caval constriction, corticosterone secretion (5 dogs) and Porter-Silber chromogen output (4 dogs) were high following midbrain transection, and subsequent bleeding failed to increase corticosterone output. It is concluded that complete midbrain transection does not interfere with the high rate of aldosterone secretion which occurs in response to acute blood loss or following caval constriction if venous pressure is maintained at the high control level.

Project IV

4a. Title: Evidence for secretion of an aldosterone stimulating hormone by the kidney.
4b. Investigators: James O. Davis, Charles C.J. Carpenter, Carlos R. Ayers, John E. Holman and Robert C. Bahn.
4c. Progress During the Year: The project is complete and the paper is in press.

Experiments were undertaken to determine the locus of secretion of the aldosterone stimulating hormone which provides the immediate stimulus to aldosterone production. In the absence of ACTH, an increase in aldosterone secretion secondary to acute blood loss was considered as presumptive evidence for secretion of ASH. In a series of successive ablation experiments, removal of the anterior pituitary, the head of the liver was followed by an increase in aldosterone secretion in response to acute hemorrhage. Following extirpation of the kidney, acute blood loss failed to effect a significant change in aldosterone secretion and the intravenous infusion of saline extracts of kidney resulted in a marked increase in aldosterone production. The data provide evidence for the renal origin of the aldosterone stimulating hormone.

Project V

5a. Title: Effects of chronic hepatic venous congestion on the disappearance of tritiated aldosterone from plasma.
5b. Investigators: Carlos R. Ayers, James O. Davis, Ferol Lieberman, and Charles C.J. Carpenter.
5c. Progress During the Year: The project is almost complete and the paper is nearly ready to go to press.

This study was undertaken to examine the possibility that chronic hepatic venous congestion results in decreased inactivation of aldosterone which contributes to the hyperaldosteronemia of congestive heart failure. Ring labeled tritiated aldosterone (2,5-5.0 uc. with a specific activity of .20-.35 uc./ug.) was injected intravenously into 7 mongrel dogs before and

after thoracic inferior vena cava (ivc) constriction which produced chronic hepatic congestion. The disappearance of authentic aldosterone from peripheral plasma was measured in 8 blood samples collected during 90 min. The biological half life ($T_{1/2}$) in 7 normal dogs ranged from 20-34 min. Five of the dogs with thoracic ivc constriction had a marked increase in $T_{1/2}$ (range = 43-128 min.) but in 2 dogs the $T_{1/2}$ was normal. The average abdominal ivc pressure was 50 mm. water higher in the 5 dogs with a prolonged $T_{1/2}$ than in the 2 dogs with a normal $T_{1/2}$. The disappearance curve after hepatectomy was almost flat indicating negligible metabolism of aldosterone in absence of the liver. The data support the view that chronic hepatic congestion contributes to hyperaldosteronemia but hypersecretion of aldosterone following caval constriction (JCI 38: 1278, 1959) was a more consistent finding than an increased $T_{1/2}$ for aldosterone.

Project VI

6a. Title: Attempts to isolate the aldosterone stimulating hormone from crude kidney extracts.
6b. Investigators: James O. Davis, Elwood Titus, Carlos R. Ayers, and Charles C.J. Carpenter.
6c. Progress During Past Year:

Saline extracts of 2 normal homogenized kidneys produced a striking increase in aldosterone secretion but no effect on corticosterone output. Heating of such crude kidney extracts to 55°C. for 10 min. denatured a lot of protein; the supernatant was assayed and found active in stimulating both aldosterone and corticosterone secretion. In another series of assays, this supernatant from the heated extracts was dialyzed and the material inside and outside the dialyzed bag were analyzed; both fractions showed activity. Also, crude kidney extracts were treated with 50% alcohol; again both the alcohol precipitated protein and the supernatant showed activity. Currently, the effects of protein precipitation with different amounts of $(NH_4)_2\ SO_4$ are under investigation.

Project VII

7a. Title: Studies on the response of the transplanted kidney and the transplanted adrenal gland to thoracic inferior vena cava constriction.
7b. Investigators: Charles C.J. Carpenter, James O. Davis, John E. Holman, Carlos R. Ayers and Robert C. Bahn.
7c. Progress During Past Year: This project is complete and paper is accepted for publication in the Journal of Clinical Investigation.

The response of a single transplanted cervical kidney to chronic thoracic inferior vena cava constriction has been investigated in six dogs. In each animal, the transplanted kidney responded to caval constriction in essentially the same manner as a normally located kidney, with virtually complete sodium retention. The response of a single transplanted cervical adrenal gland to chronic thoracic caval constriction and to superimposed acute hemorrhage has been studied in three dogs. In each animal, the transplanted adrenal gland secreted adequate aldosterone to

effect the almost complete sodium retention and the very low fecal sodium/potassium rates which is characteristic of the response of the animal with normally located adrenals to thoracic caval constriction. The transplanted adrenal gland responded to acute hemorrhage with marked increases in secretion rates of aldosterone, corticosterone and Porter-Silber chromogens. The present data demonstrate that neither intact renal and adrenal nerves nor increased renal and adrenal venous pressures are essential to the hypersecretion of aldosterone and virtually complete sodium retention which follow thoracic caval constriction. They further indicate that increased ACTH release is an important part of the response of the animal to acute hemorrhage.

Project VIII.

8a. Title: Aldosterone secretion in experimental renal hypertension with observations on the effects of renin and hypertensin II on steroid secretion.
8b. Investigators: Charles C.J. Carpenter, James O. Davis and Carlos R. Ayers.
8c. Progress During Past Year:

This experiment was designed to test the possibility that the aldosterone stimulating hormone is renin. Experimental renal hypertensin was produced by bilateral constriction of the renal arteries in dogs. A striking increase in arterial blood pressure occurred but aldosterone secretion remained normal. Extensive measurements were made during the first month of hypertension and in one animal during the third month. A large dose of renin (45 dog units) produced only slight increases in aldosterone and corticosterone secretion in the presence of marked increments in mean arterial blood pressure (from 65 to 150 mm. Hg.), at a dosage level adequate to effect a sustained increment in arterial pressure. Hypertensin II produced a variable response in both aldosterone and corticosterone secretion. The most marked increase in corticosterone secretion was associated with the smallest increment in aldosterone production. This response differed qualitatively from that produced by the kidney extracts since corticosterone failed to increase during injection of the latter. Available data suggest that renin and the aldosterone stimulating factor are different substances.

Project IX.

9a. Title: Renal origin of the aldosterone stimulating hormone in experimental secondary hyperaldosteronism.
9b. Investigators: James O. Davis, Charles C.J. Carpenter, and Carlos R. Ayers.
9c. Progress During Past Year:

In our first attempt to answer the question of the locus of secretion of ASH, the acute effect of nephrectomy on aldosterone secretion was studied in 6 dogs with chronic thoracic caval constriction and ascites; the anterior pituitary was left intact. A drop in aldosterone secretion occurred after nephrectomy in only 2 of the 6 dogs. It was reasoned that failure of aldosterone production to fall consistently following nephrectomy was due to the presence of large amounts of ACTH. Consequently, in the next experiment dogs

with hyperaldosteronism secondary to thoracic caval constriction were hypophysectomized before nephrectomy. Two or 3 days after hypophysectomy, these animals were bilaterally nephrectomized. Aldosterone secretion fell strikingly in every animal. The average decrease was from 33 to 6 m ug./min., an 82% fall. Also, corticosterone output decreased in 9 of 10 dogs; the average decrease was 81%. This degree of response in steroid secretion to nephrectomy was almost identical percentagewise to the marked fall which occurs after total hypophysectomy. This finding suggests that the low final level of secretion of aldosterone and of corticosterone is secondary to loss first of ACTH and second of ASH and that the very low rate of steroid production is the result of intrinsic biogenesis of steroids. The data are consistent with a renal origin for the aldosterone stimulating hormone in secondary hyperaldosteronism. It is quite possible that a common aldosterone stimulating hormone is secreted by the kidney in secondary hyperaldosteronism and following acute blood loss.

Project X.

10a. Title: Evidence for an hepatic humoral factor which sensitizes the kidney to aldosterone or DCA.
10b. Investigators: James O. Davis, Charles C.J. Carpenter and Carlos R. Ayers.
10c. Progress During the Year:

The animal preparation used in this experiment was a bilaterally adrenalectomized dog with thoracic caval constriction and only one kidney which was transplanted to the neck. Only one dog has been studied. The animal was given 25 mg. of DCA/day on one occasion and 1 mg./day of aldosterone on another; almost complete retention of Na occurred with both DCA and aldosterone. Following removal of the caval constriction, the dog failed to retain Na with 25 mg./day of DCA. The present working hypothesis is that the liver secretes a hormone which sensitizes the renal tubule to retain Na. Measurements are in progress to evaluate the level of GFR in the transplanted kidney in order to determine whether low GFR is a contributory factor in the presence of caval constriction.

Area II. The biochemical defect in the failing myocardium.

Project I

1a. Title: The water and electrolyte content and the contractile proteins of cardiac muscle from hypophysectomized dogs.
1b. Investigators. James O. Davis, Nicholas A. Yankopoulos and William Gay.
1c. Progress during Year:

This project is complete and the data have been partially analyzed. It is planned to complete the analysis of the data and to write the paper during 1961.

Direction of Current Research

Area I.

It is planned to continue in an effort to isolate and identify the aldosterone stimulating hormone. Studies are under way to determine 1) the specific location of the receptor which leads to release of ASH by the kidney and 2) the nature of the stimulus which activates the receptor. Finally, the possibility of a hepatic humoral factor which sensitizes the kidney to aldosterone will be pursued.

Area II.

No studies are planned in this area.

Incidental findings of significance: None

Part B.

Publications:

1. James O. Davis: Hormonal control of aldosterone secretion. Hahnemann Symposium on Edema. W. B. Saunders Co., 1960.

2. James O. Davis, Nicholas A. Yankopoulos, Ferol Lieberman, John Holman and Robert C. Bahn. The role of the anterior pituitary in the control of aldosterone secretion in experimental secondary hyperaldosteronism. J. Clin. Invest. 39: 765, 1960.

3. James O. Davis: Mechanisms of salt and water retention in congestive heart failure. Amer. J. Med. 29: 486, 1960.

4. James O. Davis, William R. Carroll, Mary Trapasso and Nicholas A. Yankopoulos: Chemical characterization of cardiac myosin from normal dogs and from dogs with chronic congestive heart failure. J. Clin. Invest. 39: 1463, 1960.

5. Nicholas A. Yankopoulos, James O. Davis, Ernest Cotlove and Mary Trapasso: Mechanism of myocardial edema in dogs with chronic congestive heart failure. Amer. J. Physiol. 199: 603, 1960.

6. James O. Davis, Charles C.J. Carpenter, Carlos R. Ayers and Robert C. Bahn: Relation of anterior pituitary function to aldosterone and corticosterone secretion in conscious dogs. Amer. J. Physiol. 199: 212, 1960.

In press:

7. Davis, James O. Mechanisms regulating the secretion and metabolism of aldosterone in experimental secondary hyperaldosteronism. Recent Progress in Hormone Research.

8. Carpenter, Charles C.J., James O. Davis, John E. Holman and Carlos R. Ayers. Response of the transplanted kidney and transplanted adrenal to thoracic inferior vena cava constriction. J. Clin. Invest.

9. Davis, James O., Carlos R. Ayers, and Charles C.J. Carpenter. Relation of the kidney to the secretion of aldosterone. Proceedings of the First International Conference on Primary Aldosteronism.

10. Davis, James O., Charles C.J. Carpenter, and Carlos R. Ayers. Evidence for secretion of an aldosterone stimulating hormone by the kidney. J. Clin. Invest.

11. Davis, James O., Evelyn Anderson, Charles C.J. Carpenter, Carlos R. Ayers, Webb-Haymaker and William T. Spence. Effects of midbrain transection and subsequent hemorrhage on aldosterone and corticosterone secretion. Amer. J. Physiol.

Serial No. NHI-252
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Halothane on Myocardial Contractile Force and Peripheral Resistance During Cardiopulmonary Bypass

Principal Investigator: Dean H. Morrow, M. D.

Other Investigator: Andrew G. Morrow, M. D.

Cooperating Unit: Department of Anesthesiology, Clinical Center

Man Years (calendar year 1960):
Total: ½
Professional: ¼
Other: ¼

Project Description: Halothane was introduced as an anesthetic agent by Raventos, et al in 1956. Since that time it has become a well established fact that in clinically useful concentrations it is capable of producing profound systemic hypotension. The etiology of this hypotension has been variously attributed to direct myocardial depression, and/or a decrease in peripheral vascular resistance. Bloodwell et al, from this Clinic, reported a study of the myocardial depressent effects of the drug as measured with the Walton-Brodie strain gauge arch. They concluded that arterial pressure did not necessarily follow the depression in myocardial activity. This study was designed to expand upon their work by assessing the central and peripheral effects of the drug physiologically separated by cardiopulmonary bypass.

Six patients undergoing routine closure of atrial septal defects under extracorporeal circulation were studied. Anesthetic premedication and maintenance operative anesthesia was selected to be well within limits previously found to have little effect on myocardial contractile force and arterial blood pressure under the conditions of the experiment. Halothane in a fixed two percent concentration was introduced into the pump oxygenator unit for a five minute period. Myocardial contractility and peripheral blood pressures were continuously recorded. Alterations in contractile force as measured with the Walton-Brodie gauge were interpreted as direct effects of the drug on the myocardium. Alterations in peripheral arterial pressure, in the presence of a constant cardiac output, were interpreted as alterations in peripheral arterial resistance.

Halothane in the stated percentage was found to decrease myocardial contractile force. This decrease was nearly linear with time, reaching -28.3% of control values during the fifth minute. Peripheral arterial resistance fell to -17% of control during the fifth minute, this rate of fall being slower than

that of contractile force for similar times of exposure. With cessation of drug administration each parameter returned toward control values. It was concluded that the hypotensive effects of halothane may be attributed to two distinct physiologic actions of the drug.

Part B included Yes.

Serial No. NHI-252

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Bloodwell, R. D., Brown, R. C., Christenson, G. R., Goldberg, L. I., and Morrow, A. G.: The effect of Fluothane on myocardial contractile force in man. Anesthesia and Analgesia--Current Researches. In Press.

Serial No. NHI-253
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: An Increased Incidence of Paradoxical Hypertension Following Resection of Coarctation of the Aorta Under Halothane Anesthesia

Principal Investigator: Thomas B. Davis, M. D.

Other Investigators: Dean H. Morrow, M. D.
Theodore Cooper, M. D.
Clarence L. Hebert, M. D.

Cooperating Units: Department of Anesthesiology, Clinical Center

Man Years (calendar year 1960):
Total: 1
Professional: ½
Other: ½

Project Description: Sealy and others have reported that paradoxical hypertension occurs in about 10% of patients who undergo surgical correction of aortic coarctation. This same incidence has been noted at the National Heart Institute in the past few years. However, during this past year five consecutive patients that underwent surgical resection of aortic coarctations developed postoperative hypertension. The one obvious difference in the handling of these five patients was that all received a new anesthetic agent, halothane.

Because halothane is known to have profound cardiovascular effects we elected to study this agent in relationship to the postoperative hypertension seen in those patients that had repairs of aortic coarctation.

Five mongrel dogs were studied, each dog twice. On one occasion nitrous oxide thiopental was the anesthetic and on another occasion nitrous oxide halothane was employed. Continuous intra-arterial pressures were measured before, during, and after an acute total occlusion of the thoracic aorta. After the chest was closed, the anesthetic gases were discontinued and arterial pressures monitored for one hour post anesthesia.

The results showed a definite tendency for postoperative hypertension to occur in the five animals that received nitrous oxide halothane anesthesia. Whereas in the five animals that received nitrous oxide thiopental the average pressures at one hour postanesthesia, postocclusion did not exceed the average control pressures.

Proposed Course of Project: The observations noted suggested a study of renal blood flow during anesthesia with these mixtures and this project is described separately.

Part B included No.

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Serial No. NHI-254
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Prosthetic Heart Valves

Principal Investigator: Nina S. Braunwald, M. D.

Other Investigator: Andrew G. Morrow, M. D.

Man Years (calendar year 1960):
Total: 5
Professional: 2
Other: 3

Project Description: Work has been in progress over the past year on the development of a prosthetic mitral valve to replace those valves severely deformed by disease. A flexible plastic foam valve has been developed and tested on a pulse duplicator and in a series of dogs. After demonstration of competency of this valve in the laboratory, it has been used in patients severely incapacitated by mitral insufficiency. One patient whose mitral valve was completely replaced by a prosthetic polyurethane foam valve survived for over three months following surgery and demonstrated adequate valve function. No demonstrable clotting of the valve or systemic embolization was evident at subsequent autopsy examination.

Proposed Course of Project: Continued efforts are being made to improve the material and molding techniques used in the fabrication of these artificial valves so that function will more closely approximate that of a normal valve. In addition, the techniques used in the development of an artificial mitral valve are now being applied to the development of prosthetic aortic valves and individual aortic leaflets.

Part B included Yes.

Serial No. NHI-254

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Braunwald, N. S., Cooper, T. and Morrow, A. G.: Complete replacement of the mitral valve. J. Thor. and Cardiovas. Surg. 40:1-11, 1960.

Braunwald, N. S., Cooper, T., and Morrow, A. G.: Experimental replacement of the mitral valve with a flexible polyurethane foam prosthesis. Trans. Amer. Soc. Artif. Inter. Organs. 6:312-322, 1960.

Serial No. NHI-255
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Prosthetic Replacement of the Pulmonary Artery in Dogs

Principal Investigator: Joseph W. Gilbert, M. D.

Other Investigators: Theodore Cooper, M. D., Ph. D.
Paul A. Ebert, M. D.

Man Years (calendar year 1960):
Total: 1½
Professional: ½
Other: 1

Project Description: Anatomic correction of certain congenital cardiac malformations, such as severe tetralogy of Fallot is predicated upon replacement of the main pulmonary artery. In a previous study of unilateral pulmonary artery replacement, the use of crimped teflon vascular prostheses in the lesser circulation of dogs was explored and found feasible (1).

In an extension of this study, valveless vessel prostheses ½" - 3/4" in diameter, 4-6 cm. in length, were attached to the main pulmonary artery (end-to-side anastomosis) and inserted into the outflow tract of the right ventricle during brief caval occlusion. The main pulmonary artery was thereupon interrupted by ligature..

Eight animals survived the procedure for periods of 1 to 5 months and five are still living. Right heart catheterization, cardiac outputs(Fick) and selective angiocardiograms have been carried out in these dogs at intervals of two weeks and three months. All have shown marked elevation in right ventricular pressures (40-60 mm. Hg) and substantial gradients across the valveless prostheses. Five animals died at intervals of 3 days to one month, with postmortem findings characteristic of right heart failure.

Several observations have thus far been made in this experience: Stricture of anastomoses, as seen in prosthetic caval replacement and thromboses, as seen in homograft replacement of the pulmonary artery have not been encountered. The significance of the length of the rigid graft in imposing an increased resistance in the lesser circulation has been impressive. This has doubtless been amplified by the increased stroke volume required of the right ventricle in the face of complete absence of a pulmonary valve. The cardiac outputs, however, at postoperative intervals up to 3 months, have been normal.

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Proposed Course of the Project: Replacement of the main pulmonary artery during brief caval occlusion, has necessitated end-to-side anastomosis of the prosthesis and hence a graft of excessive length has been required. Complete cardiopulmonary bypass, permitting end-to-end anastomosis of graft to pulmonary artery, should permit insertion of a shorter prosthesis, and hence a more tolerable insult.

Part B included Yes.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Gilbert, J. W., Cornell, W. P., and Cooper, T.: An experimental study of pulmonary artery replacement. J. Thoracic Surg. In Press.

Serial No. NHI-256
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Cardiac Implantation Evaluation of Plastic Materials Used in the Fabrication of Prosthetic Heart Valves

Principal Investigator: William C. Awe, M. D.

Other Investigators: Nina S. Braunwald, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1960):
Total: 1
Professional: ½
Other: ½

Project Description: The feasibility of mitral valvular replacement has been demonstrated but the fate of the plastic materials used in the fabrication of these valves has not been determined. The purpose of this project is to evaluate these materials in the intracardiac environment. The materials must be durable enough to withstand the stress and strain applied to them by years of cardiac action, must not promote intracardiac clotting, must not calcify and must not become replaced by ingrowth of tissue. Intracardiac implantation should answer these questions.

Plastic materials are implanted in the right atrium of dogs by applying a noncrushing clamp to the segment of the atrium into which the plastic material is to be implanted. At intervals of 3 months, 6 months, and one year, the animals will be sacrificed and the implants examined for tissue ingrowth, clotting, and calcification. Tensile strength will be measured. The materials showing the most promise will be used to manufacture prosthetic valves.

Part B included No.

Serial No. NHI-257
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Evaluation of Artificial Chordae Tendinae in the Canine Heart

Principal Investigator: William C. Awe, M. D.

Other Investigators: Nina S. Braunwald, M. D.
Andrew G. Morrow, M. D.

Man Years: (Calendar year 1960)
Total: ½
Professional: ¼
Other: ¼

Project Description: The prosthetic mitral valves which have been constructed in the laboratory all utilize prosthetic chordae tendinae which prevent the eversion of the valve leaflets into the left atrium during ventricular systole. The artificial chordae tendinae course through the ventricular cavity and tend to promote clotting. A second problem concerns the attachment of the chordae tendinae to the ventricular surface. In an effort to determine which materials are most suitable for the fabrication of chordae tendinae, the present study was undertaken. Various methods of attaching the chordae tendinae to the ventricular surface are being evaluated.

A long curved needle is used to pass the material from the posterior surface of the left ventricle, through the left ventricular cavity, through the interventricular septum and on to the anterior surface of the heart. The materials being tested are wire of various sizes, silk, silk coated with polyurethane and teflon. Dogs will be sacrificed at three months, six months, and one year and the materials and methods of attachment will be evaluated.

Part B included No.

Serial No. NHI-258
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Supportive partial cardiopulmonary bypass: Distribution of perfusion flow

Principal Investigator: Joseph W. Gilbert, M.D.

Other Investigators: Paul Ebert, M.D. and Lazar Greenfield, M.D.

Man years (calendar year 1960):
Total: ½
Professional: ½
Other: 0

Project Description: Experimental study of partial bypass including veno-arterial perfusion has been carried out in several laboratories. Although many problems are associated with this method of support of a failing heart, it has been shown that the work of the heart is reduced by partial (femoral vein-artery) bypass and that coronary perfusion is thereby improved. Metabolic acidosis has, however, been associated with such technics, requiring the administration of bicarbonate. The distribution of a metered partial perfusion, as determined by direct measurement of portal, renal, hepatic, and opposite extremity blood flow should thus be of significance.

Adult mongrel dogs, anesthetized and respired mechanically, are to be subjected to partial bypass, employing gravity drainage from one femoral (or iliac) vein and metered perfusion of the ipsilateral femoral artery. Selective exclusion of venous flows from the various beds may be directly measured by appropriate caval cannulation and pertinent chemical determinations obtained. Similar studies are to be carried out on animals subjected to simple veno-arterial perfusion and perfusion with an oxygenator.

Proposed Course of Project: Insofar as this study has not yet been implemented, detailed outline of various problems which may be encountered is not possible.

Part B included No.

Serial No. NHI-259
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Artificial Constriction of the Pulmonary Artery: Clinical results following operation in a series of infants with ventricular septal defect and pulmonary hypertension.

Principal Investigator: Nina S. Braunwald, M. D.

Other Investigator: Andrew G. Morrow, M. D.

Man Years (calendar year 1960):
Total: 1
Professional: ½
Other: ½

Project Description: The operative mortality for complete repair of ventricular septal defect remains high in young children who develop symptoms of severe congestive heart failure early in life. Artificial production of pulmonic stenosis by surgical constriction of the pulmonary artery should produce hemodynamic cardiovascular changes which benefits this group of patients.

A clinical evaluation of a group of 16 patients who have undergone pulmonary constriction is being carried out. The operative mortality for children with an isolated ventricular septal defect and pulmonary hypertension has been 7.7%. The improvement in the physical development, exercise tolerance and the decrease in incidence of pulmonary infection is clearly evident following pulmonary constriction. The results of this study thus far suggests that continuation of this operative procedure and further study of the course of this group of patients is indicated.

Part B included No.

Serial No. NHI-260
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Cardiac Response to Digitalis Following Extracorporeal Circulation

Principal Investigator: Paul A. Ebert, M. D.

Other Investigators: Lazar Greenfield, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar Year 1960):
Total: ½
Professional: ¼
Other: ¼

Project Description: Most patients undergoing open cardiac surgery with the use of extracorporeal circulation have previously been receiving some type of digitalis preparation. Postoperatively the amount of digitalis necessary for maintenance has been a problem since the effects of cardiopulmonary bypass on the response and digitalis content of the heart has been left to clinical judgment.

Dogs were given a standard dose of digoxin and then titrated to either ventricular tachycardia or complete heart block with acetylstrophanthidin. These dogs were again given their dose of digoxin on a subsequent day, put on cardiopulmonary bypass of 30 min. and after bypass again titrated with acetylstrophanthidin. Each dog served as his own control. It was found that the amount of acetylstrophanthidin required to produce tachycardia or block after cardiopulmonary bypass was the same as the control dose. It was concluded that extracorporeal circulation has no demonstrable effect on the effective quantity of digitalis present in the myocardium.

Proposed Course of Project: Further studies are planned using tritium tagged digoxin to analyze the quantity of radioactive digoxin present before and after cardiopulmonary bypass.

Part B included No.

Serial No. NHI-261
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Experimental Production of Coronary Artery-Right Atrial Fistula

Principal Investigator: Paul A. Ebert, M. D.

Other Investigators: Lazar Greenfield, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1960):
Total: 1½
Professional: ½
Other: 1

Project Description: The rarity of congenital coronary-right atrial fistula in humans has often led to incorrect diagnosis. The lesion has been created in dogs by implanting the open end of the right coronary artery into the right atrium. These animals are to be kept for 6 months and studied by 1) right heart catheterization; 2) dye dilution curves; and, 3) aortography. The animals will then be sacrificed and a detailed pathologic study of the lesion made. Of particular interest will be measurements of the size of the artery proximal to the fistula. If pulmonary hypertension develops in these animals this will also be further studied.

It is hoped that the information obtained will lead to more definite criteria for the diagnosis of this lesion in humans and also elucidate the mechanism of arterial dilatation in A-V fistula.

Part B included No.

Serial No. NHI-262
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effects of Renal Perfusion of Hemolyzed Blood on Renal Function in the Dog

Principal Investigator: Lazar J. Greenfield, M. D.

Other Investigator: Paul A. Ebert, M. D.

Man Years (calendar year 1960):
Total: 3
Professional: 1
Other: 2

Project Description: The occasional complication of oliguria or anuria following prolonged cardiopulmonary bypass in humans has been thought to be associated with a toxic effect of damaged erythrocytes. Although a high level of free plasma hemoglobin is frequently present following long perfusions, there is poor correlation with postoperative oliguria and free hemoglobinemia has been shown to be well tolerated in humans.

In the present study PAHA and creatinine were utilized to measure renal plasma flow and glomerular filtration rate in mongrel dogs. Following control studies, the left kidney is excised and the right renal vessels are cannulated from the left side. Perfusion for one hour with a small bubble oxygenator apparatus at flows of 70-100 cc./min. is performed with varying amounts of hemolyzed dog blood added to the perfusate. Followup renal function studies are done on the 2nd day, and serial BUN determinations are made. The dogs are sacrificed at seven days and the kidneys subjected to pathological analysis.

Proposed Course of Project: This method of unilateral renal perfusion with chronic survival appears to hold promise for evaluation of a variety of drugs as well as different conditions of perfusion. It is hoped that the present study will shed some light on the problem of post-perfusion oliguria.

Part B included No.

Serial No. NHI-263
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Measurements of Blood Pressure and Regional Blood Flow during Profound Hypothermia

Principal Investigator: Paul A. Ebert, M. D.

Other Investigators: Lazar Greenfield, M. D.
Andrew G. Morrow, M. D.
Joseph W. Gilbert, M. D.

Man Years (calendar year 1960):
Total: 3
Professional: 1
Other: 2

Project Description: With the increased use of profound hypothermia during operations on the heart or brain it has become important to know the changes in circulation to various areas of the body associated with changes in temperature. By the use of extracorporeal circulation, maintaining a constant arterial flow to the animal, and the temperature regulated by a Pemco heat exchanger located in the arterial line, the temperature of the animal could be regulated.

Constant monitoring of the mean arterial pressure shows that the peripheral resistance falls with temperature change to 10°C. With this change in resistance it is of interest to determine what change in blood flow occurs through various regions of the body.

Carotid blood flow decreases with the decrease in mean arterial pressure while femoral arterial flow increases. Preliminary studies would indicate that renal blood flow remains relatively constant and that blood flow through the liver decreases although a rise in portal venous pressure occurs.

Proposed Course of Project: To continue the measurement of renal, hepatic, and femoral artery blood flow in dogs undergoing complete cardio-pulmonary bypass and profound hypothermia. More extensive study of the blood flow of the liver during hypothermia is planned.

Part B included No.

Serial No. NHI-264
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Large Doses of Quinidine and Procaine Amide on Myocardial Function in the Normothermic and Hypothermic Dog

Principal Investigator: E. Kent Carney, M. D.

Other Investigators: John Ross, Jr., M. D.
Theodore Cooper, M. D., Ph. D.

Man Years (calendar year 1960):
Total: 1½
Professional: ½
Other: 1

Project Description: It has been previously shown that large doses of quinidine severely depress myocardial function in the normothermic dog, and that moderate hypothermia in part mitigates this depression. These studies were carried out in dogs utilizing 3 techniques:

1. Evaluation of "left ventricular function curves," determined by plotting left ventricular stroke work against left ventricular filling pressure, demonstrated decreased work capacity of the heart following quinidine;

2. The Walton-Brodie strain gauge arch sutured directly to the left ventricular myocardium gave direct evidence of depression of myocardial contractile force following quinidine administration;

3. The heart was excluded from the system incorporating a heart-lung machine, and quinidine produced a marked fall in mean arterial pressure at a constant inflow rate of blood, and moreover there was an increase in total intravascular volume, indicating a fall in total peripheral vascular resistance.

Proposed Course of Project: Further studies will be carried out to determine the effects of procaine amide by these techniques. In addition, the changes produced by profound hypothermia will be studied. When it has been determined that administration of these agents is safe in the experimental animal, the study will be extended to human subjects undergoing thoracic surgery.

Part B included No.

Serial No. NHI-265
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: An Unusual Lesion of Synovial Vasculature

Principal Investigator: Leon Sokoloff, M. D.

Other Investigator: Stanley M. Elmore, M. D.

Cooperating Units: National Institute of Arthritis and Metabolic Diseases

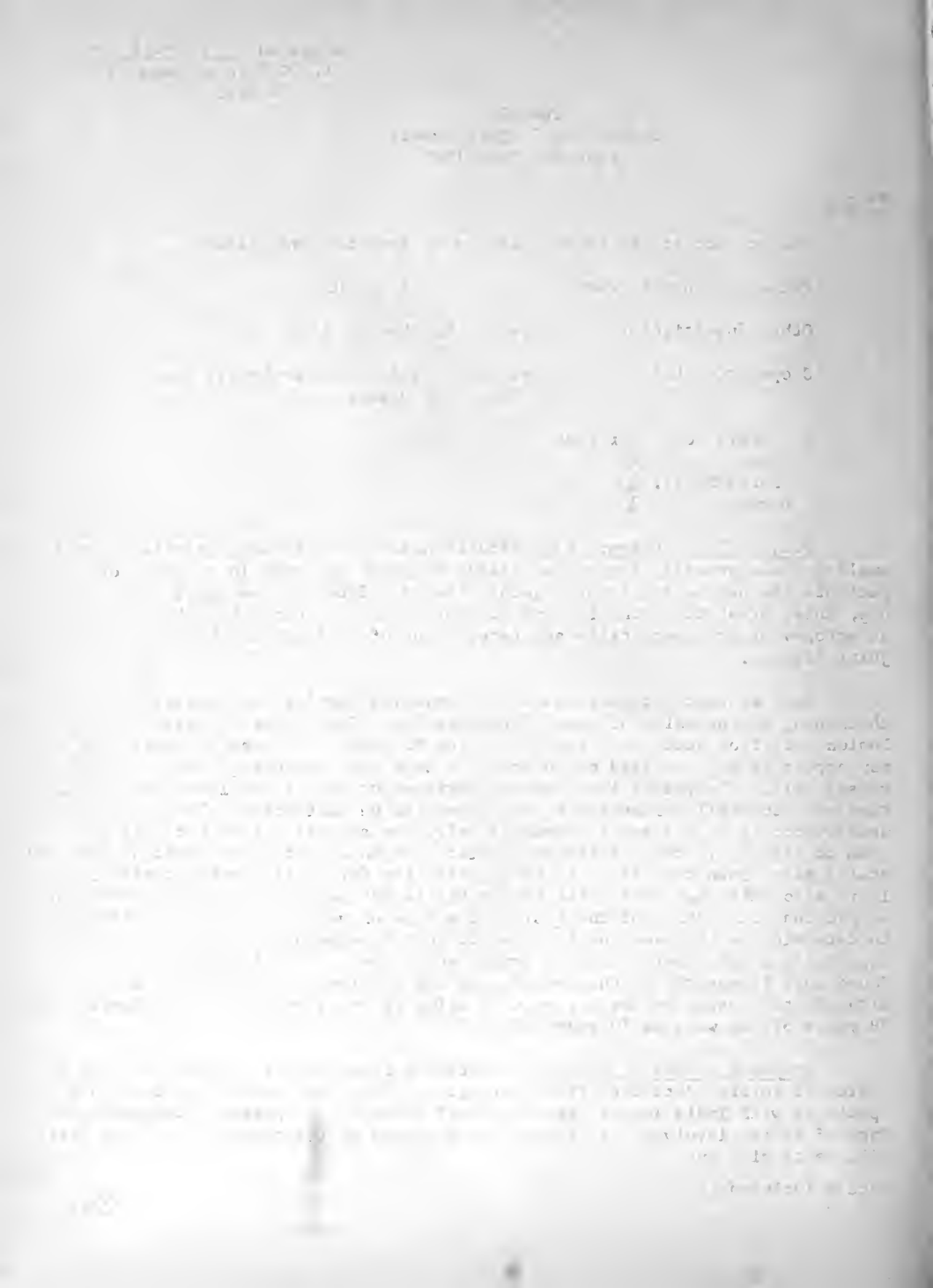
Man Years (calendar year 1960):
Total: ½
Professional: ¼
Other: ¼

Project Description: A previously undescribed lesion, peculiar to the smaller blood vessels of synovial tissue has been observed in a series of patients who had no symptomatic joint disease. Synovial specimens of the hip, knee, great toe, fingers, and sterno-clavicular joints have been taken at autopsy or from surgically amputated limbs of patients who had no clinical joint disease.

Some microscopic sections of the snyovial samples have revealed a thickening of the walls of some of the vessels. The characteristics of the lesion vary from section to section, joint to joint and among patients. It may appear as an increased cellularity or as a hyalinized thickening of the vessel wall. The vessel lumen may be narrowed or totally occluded in association with the wall thickening or may appear to be unaffected. The lesion usually occurs in the smaller vessels below the synovial layer but does not seem to affect all the vessels of similar size in a particular section. Special stains have shown the lesion to be negative for fat, ruling out arteriosclerosis. It is also deficient in elastic tissue but is PAS positive. Due to the microscopic characteristics of the lesion, the type of vessel affected is difficult to determine as the synovium is known to contain elements of arteries, veins, capillaries, and complex arterio-venous anastomosis. The lesion has been found most frequently in the synovium of the pulvinar acetabulum and knee. Although the lesion may be a result of aging it has been found in patients 38 years old as well as 70 years old.

Proposed course of project: Specimens from younger patients will be collected to elucidate the effects of aging. Injection studies of the gross specimens with India Ink or other contrast media are proposed to determine the type of vessel involved. A statistical analysis of the occurrence of the lesion will be carried out.

Part B included No.



Serial No. NHI-266
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Determination of the Hematocrit of the Systemic Circulation of Humans During Total Body Perfusion

Principal Investigator: Roland J. Folse, M. D.

Other Investigator: Eugene Braunwald, M. D.

Man Years (calendar year 1960):
Total: ½
Professional: ½
Other: 0

Project Description: There appears to exist a discrepancy between the total blood volume, as calculated from the albumin space and large vessel hematocrit, and the total blood volume calculated from the red cell volume and large vessel hematocrit. This is partially related to the fact that the splanchnic to large vessel hematocrit ratio is less than one. An additional factor may be the relatively slower circulation of albumin in the capillary bed. This experiment is designed to measure the mean transit times of Cr^{51} tagged red cells and I^{131} serum albumin when injected simultaneously into the systemic circulation of patients on cardiopulmonary bypass for open heart surgery. The systemic circulation hematocrit can then be calculated using the method of Lilienfield et al and compared to the large vessel hematocrit.

Proposed Course of Project: I^{131} serum albumin will be obtained from the Squibb Laboratories, prepared for human injection, in 0.25 mc. quantities to be shipped on Mondays. $Na_2Cr^{51}O_4$ will be obtained from the Squibb Laboratories, prepared for human use, in 1.0 mc. quantities. Red cells from the blood bank, previously cross-matched with the patient's cells, will be tagged with $Na_2Cr^{51}O_4$. These cells will be mixed with I^{131} serum albumin to give a sterile injectate containing 25 to 50 μc. of Cr^{51} and 20 to 40 μc. of I^{131}. This will be injected into the arterial line of the pump oxygenator and will circulate through the systemic circulation of the patient. Two to five second samples will be collected from the venous return line over a one minute period following injection. The plasma and red cells will be separated by centrifugation. The red cells will be washed, and aliquots of plasma and red cells placed in vials will be counted using a scintillation counter. The counts will be plotted against time using the Stewart-Hamilton principle. Red cell and plasma flows, mean circulation times, and systemic hematocrit can then be calculated.

Part B included No.

Serial No. NHI-268
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: An Experimental Method for the Production of Pulmonary Hypertrophic Osteoarthropathy

Principal Investigator: Stanley M. Elmore, M. D.

Other Investigators: Leon Sokoloff, M. D.

Cooperating Units: National Institute of Arthritis and Metabolic Diseases

Man years (calendar year 1960):
Total: ½
Professional: ¼
Other: ¼

Project Description: Pulmonary hypertrophic osteoarthropathy has been experimentally produced on only one occasion. Mendlowitz reported the occurrence of pulmonary hypertrophic osteoarthropathy in one of four dogs in which a fistula between the left pulmonary artery and left atrium was created surgically. Confirmation of this experiment has never been reported nor has any other method of experimental production of this osseous lesion.

The occurrence of pulmonary hypertrophic osteoarthropathy arising secondary to a variety of fatal and nonfatal clinical diseases has been reported but the pathogenesis of pulmonary hypertrophic osteoarthropathy has remained obscure and has led to a multiplicity of theories as to its origin.

Eighteen dogs have been subjected to one of several operations designed to produce arterial oxygen unsaturation and presumably pulmonary hypertrophic osteoarthropathy. The operation of Mendlowitz has been repeated in several dogs, resulting in an arterial O_2 saturation of 89%. If the left pulmonary artery-left atrial shunt was combined with total ligation of the left pulmonary artery distal to the fistula or transection of the left main stem bronchus the arterial O_2 saturation was reduced to approximately 80%. These combined operations have been done in several animals. Also the superior vena cava was anastomosed to the left atrium in one dog with a resultant arterial O_2 saturation of 78%.

Proposed Course of Project: The present series is to be enlarged by submitting more animals to the aforementioned operations and by trying other methods which show promise of producing pulmonary hypertrophic osteoarthropathy experimentally.

Part B included No.

Serial No. NHI-269
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Mechanism of the Inotropic, Chronotropic and Pressor Effects of Guanethidine and Bretylium

Principal Investigator: Thomas E. Gaffney, M. D.

Other Investigators: Eugene Braunwald, M. D.
Theodore Cooper, M. D., Ph.D.

Man Years (calendar year 1960):
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description: Guanethidine and bretylium are two drugs used clinically as hypotensive agents. Paradoxically, their intravenous injection produces a transient pressor response and a positive inotropic and chronotropic effect. The mechanism of these effects has not been adequately defined. We have utilized cardiac denervation with resultant myocardial catecholamine depletion to provide an otherwise intact animal in which the pressor, inotropic and chronotropic effects of guanethidine and bretylium may be measured and subsequently compared to responses in normal dogs and to the data we had previously obtained in isolated, catecholamine depleted hearts.

The pressor, inotropic (strain gauge arch) and chronotropic effects of guanethidine and bretylium (.3 mg. - 10 mg./Kg.) have been measured at least once in each of 4 dogs with chronically denervated hearts and in 8 normal dogs. The positive inotropic and chronotropic effects of guanethidine in the normal dog are absent in the dog with an areflexic, catecholamine depleted heart. Instead, in 3 of 4 of these denervated preparations guanethidine had a negative instead of positive inotropic effect. The pressor response to guanethidine was greatly diminished or absent in each denervated preparation. Bretylium produced a marked positive inotropic, chronotropic and pressor effect in each normal dog and in the denervated preparation only the chronotropic effect was reversed.

These effects upon heart rate and contractile force are qualitatively identical to observations we had previously made in the isolated, catecholamine depleted heart and support the hypothesis that guanethidine produces a positive inotropic and chronotropic effect by myocardial catecholamine release whereas bretylium has an intrinsic positive inotropic effect. Furthermore, these effects produced by sudden myocardial amine release are a striking illustration of the physiological potential of stored myocardial amines.

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Individual Project Report
Calendar Year 1960

Proposed Course of Project: 1) measurement of changes in cardiac output produced by guanethidine and bretylium in the normal and cardiac denervated dog, and 2) use of the cardiac denervated preparation for a series of physiological and pharmacological studies designed to measure the importance of intrinsic myocardial catecholamines in physiological and drug responses.

Part B included No

853

Serial No. NHI-270
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Syrosingopine on the Cardiac Response to Acute Hypoxemia and Exercise

Principal Investigator: Charles Chidsey, M. D.

Other Investigators: Robert L. Frye, M. D.
Richard L. Kahler, M. D.
Eugene Braunwald, M. D.

Man Years (calendar year 1960):
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description: Because studies using preparations of experimental animals have indicated that rauwolfia alkaloids can produce functional denervation of the cardiovascular system, it was of interest to see if these drugs would alter cardiovascular responses in unanesthetized man. A semisynthetic rauwolfia alkaloid, syrosingopine was given parenterally to 5 subjects over a period of 7 days. The average total amount given was 0.5 mg./Kg., sufficient to produce orthostatic hypotension in each case.

Observations were made of the cardiovascular response to acute hypoxemia induced by the inhalation of 12% oxygen before administration of syrosingopine and after 7 days on the medication. The average increment in cardiac output during the control period was essentially unchanged by the administration of syrosingopine. There was, however, a smaller pulse rate increment response to hypoxemia without syrosingopine. The blood pressure was reduced after drug treatment, but none of the other variables were altered. A similar study was done substituting exercise as the stimulus. Here again the response was unchanged by syrosingopine.

The therapeutic implication of these findings would be that rauwolfia alkaloids even in large amounts do not interfere with the ability of the heart to meet certain demands placed upon the cardiovascular system. The persistent ability to respond in the face of presumed norepinephrine depletion in the heart suggests that either the residual transmitter substance remaining is able to effect the stimulation or that factors other than the cardiac sympathetic nerves are involved. Further work will be required to elucidate this problem.

Proposed Course of Project: Completed

Part B included No 1

Serial No. NHI-271
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Measurement of Left Ventricular Volume by Biplane Angiocardiography

Principal Investigator: William L. Gleason, M. D.

Other Investigator: Eugene Braunwald, M. D.

Man Years (calendar year 1960):
Total: 0.5
Professional: 0.3
Other: 0.2

Project Description: An effort is being made to utilize the method of Dodge et al for the measurement of the left ventricular volume. Simultaneous antero-posterior and lateral films of the chest are made at the rate of 6/sec. following the injection of contrast material directly into the left ventricle. The appropriate dimensions taken from the antero-posterior and lateral projections of the ventricular outline are corrected for non-parallel x-ray distortion and inserted in an elipsoid formula for calculation of ventricular volume. A photocell is used to record the time relations of each pair of films with the electrocardiogram and the brachial arterial pressure tracing. By selecting end-diastolic and end-systolic films and subtracing the ventricular volumes derived from these films it is possible to calculate stroke output and to relate this to the preceding end-diastolic volume. By comparing the total stroke output of the ventricle determined in this way with the "effective" stroke output determined from the indicator dilution curve it should be possible to quantitate either mitral or aortic regurgitation.

To date we have demonstrated a good correlation between the angiocardiographic and the dye dilution stroke outputs (agreement within 15%) in three patients without significant regurgitation. In one patient with auricular fibrillation and one with pulsus alternans we have shown a good correlation between end-diastolic volume and stroke volume.

Proposed Course of Project: 1) the method must be further validated by a) securing more comparisons between the angiocardiographic and dye dilution stroke output, b) comparison of the volume of a left ventricular plaster of paris cast (post mortem human heart) determined by the angio method with the volume determined by displacement, 2) the method can be used to determine the degree of valvular insufficiency in patients with disease of mitral or aortic valves and the clinical value of this method must be assessed, 3) it should

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Part I

be possible to determine the relation between end-diastolic volume and stroke volume under a variety of circumstances, and 4) an absolute measure of the volume of the left ventricle should permit the calculation of the tension, total force and amount of shortening of the left ventricular muscle.

Part B included No

Serial No. NHI-272
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Clinical Significance of the First Derivative of the Left Ventricular Pressure Pulse

Principal Investigator: William L. Gleason, M. D.

Other Investigators: Eugene Braunwald, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1960):
Total: 0.5
Professional: 0.3
Other: 0.2

Project Description: An electronic differentiating circuit has been assembled and a means of calibrating this instrument devised. With this equipment it is possible to obtain a continuous recording of the rate of change of the left ventricular pressure. At present, the study has been limited to the factors influencing maximum rate of pressure rise in the left ventricle.

In twelve patients left ventricular pressure contours have been recorded by means of a direct needle puncture of the left ventricle, either open chested in the operating room or percutaneously during diagnostic studies. Six of these patients have had mitral valve disease, three patent ductus and three aortic stenosis. The normal peak derivative is approximately 1200 mm.Hg/sec. With aortic stenosis or insufficiency this value is greatly increased often to levels of 2000 to 3000 mm.Hg/sec. In patients with atrial fibrillation the peak derivative of individual beats varies considerably. In these patients the peak derivative shows a good correlation with the following variables: 1) pulse pressure, 2) end-diastolic pressure, 3) duration of preceding diastole, and 4) duration of ejection. In one patient with a patent ductus and coarctation of the aorta it was noted that clamping of the ductus and therefore decreasing left ventricular output, produced a distinct decrease in the peak derivative even though the ventricular systolic pressure increased. These observations are consistent with the hypothesis that there is a fundamental relation between the peak derivative of the left ventricular pressure pulse and the end-diastolic volume.

Proposed Course of Project: 1) further studies will be necessary in patients with normal myocardium and normal aortic valves to define more precisely the normal range of the peak pressure derivative and to determine to

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what extent it may be influenced by factors which alter stroke output or arterial pressure; 2) further studies are needed to determine whether the peak derivative can be related to the severity of aortic stenosis or insufficiency, and 3) patients with myocardial disease and left ventricular dilatation must be studied to determine whether the peak derivative is diminished by myocardial weakness.

Part B included No

Serial No. NHI-273
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Guanethidine on Triiodothyronine Induced Hyperthyroidism in Man

Principal Investigator: Thomas E. Gaffney, M. D.

Other Investigators: Eugene Braunwald, M. D.
Richard L. Kahler, M. D.

Man Years (calendar year 1960):
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description: The mechanisms underlying the cardiovascular manifestations of hyperthyroidism are not understood. The contribution of catecholamine potentiation by thyroid hormone is uncertain. Reserpine has been shown to reduce the tachycardia of hyperthyroidism and this effect is probably related to myocardial catecholamine depletion.

The availability of guanethidine which reduces tissue amines and interferes with reflex release of catecholamines at the adrenergic neuroeffector site provides an opportunity to estimate indirectly, the contribution of catecholamines to the hyperthyroid syndrome.

Triiodothyronine (250 ug./day) given to two young normal control subjects produced an increase in sleeping and resting pulses, systolic and diastolic blood pressure, tremor, elevations of the basal metabolic rate and a decrease in serum cholesterol. Guanethidine (30 - 60 mg./day) given to both subjects after the above changes (during continued triiodothyronine administration) produced a marked decrease in sleeping and resting pulses, palpitation, hand tremor and blood pressures. The increased basal metabolic rate and decreased serum cholesterol persisted unchanged during guanethidine treatment. Placebo substitution for guanethidine produced a return of all parameters toward preguanethidine levels in both subjects.

Proposed Course of Project: 1) increase number of subjects studied to four, 2) possible trial use of guanethidine in treatment of hyperthyroidism, and 3) a comparison of changes in cardiac output, blood pressure and heart rate induced by thyroid administration in normal dogs and dogs with cardiac denervation (with resultant myocardial catecholamine depletion). This study would estimate the contribution of cardiac reflexes and myocardial catecholamines specifically, to the cardiovascular changes of hyperthyroidism.

Serial No. NHI-274
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Hypothermia on the Arterial and Venous Beds in the Dog

Principal Investigator: Richard L. Kahler, M. D.

Other Investigators: Allan Goldblatt, M. D.
Eugene Braunwald, M. D.

Man Years (calendar year 1960):
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description: Hypothermia has been produced in eight dogs utilizing complete cardiopulmonary bypass with heat exchangers incorporated into the circuit. Cooling has been effected to 15 - 25° C. During the cooling phase a decrease in peripheral vascular resistance (app. 30%) and an augmentation of intravascular blood volume have been noted. Venous tone as measured by intermittent venous occlusion technique has revealed venodilatation during this phase. Upon rewarming to 37° C arteriolar resistance increased to slightly above control values and the intravascular blood volume decreased. These studies reveal arteriolar and venous dilatation during deep hypothermia with reversal of these changes with rewarming. These changes have not been affected by previous adrenalectomy.

Proposed Course of Project: Studies are planned to further quantitate these changes and to determine the mechanisms which effect these changes.

Part B included No

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Part VII

Independent Project Report

Electronic Circuit Design

Part 2

Serial No. NHI-275
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Hypoxia and Hypercapnea on the Venous Bed

Principal Investigator: Richard L. Kahler, M. D.

Other Investigators: Allan Goldblatt, M. D.
Eugene Braunwald, M. D.

Man Years (calendar year 1960):
Total: 0.5
Professional: 0.3
Other: 0.2

Project Description: The effects of hypoxia and hypercapnea on the capacity of the total systemic vascular bed, on venous distensibility and venous return were studied in an experimental preparation in which the use of extracorporeal circulation permitted complete hemodynamic separation of the central and systemic circulations. Alterations of intravascular blood volume were measured continuously. Brief occlusions of the venous outflow line permitted inscription of venous pressure-volume curves. Right ventricular contractile force was measured with the Walton-Brodie strain gauge arch. Hypoxia (5-10% O_2) in 12 dogs produced venoconstriction and a decrease in intravascular blood volume averaging 17.0 ml./Kg. Hypercapnea (6% CO_2) produced a similar but quantitatively smaller (av. 9.9 ml./Kg.) response. Alterations in venous distensibility were consistent with the observed changes in vascular capacity. Qualitatively similar changes occurred with hypoxia following bilateral adrenalectomy and splenectomy. Hypoxia produced an increase in right ventricular contractile force (av. 36%) and a decrease in peripheral vascular resistance (av. 11%). During hypercapnea right ventricular contractile force decreased (av. 27%) as did total peripheral vascular resistance (av. 9%). These experiments indicate that hypoxia and hypercapnea produce venoconstriction and that the hypertension which occurs with hypoxia in the intact animal is probably due to increased cardiac output rather than increased peripheral vascular resistance.

Proposed Course of Project: Further studies are planned utilizing differential perfusion techniques to elucidate the mechanism behind these changes.

Part B included No

Serial No. NHI-276
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Character of Right Ventricular Conduction Disturbance Following Right Ventriculotomy

Principal Investigator: Allan Goldblatt, M. D.

Other Investigators: Eugene Braunwald, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1960):
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description: The electrocardiographic findings of right bundle branch block following right ventriculotomy has been described by numerous investigators. The etiology of the development of the right bundle branch block has been attributed to the right ventriculotomy by some, whereas other workers have felt that it is the result of operative damage in the regions involving the crista supraventricularis.

There have been no known reports in the literature discussing the time relationship between the post-operative prolongation of the QRS complex with right ventricular conduction delay and the onset of electro-mechanical contraction of the right ventricle. If with a post-operative prolongation of the QRS complex, there is a concomitant delay in the onset of right ventricular electromechanical contraction, then one can state that this is true right bundle branch block. If, however, there is no delay in the onset of right ventricular electro-mechanical contraction, then one can assume that the conduction defect is a parietal block, rather than true right bundle branch block.

It was with this thought in mind that the following study was undertaken. Thus far the preoperative and postoperative catheterization records of 13 patients having undergone right ventriculotomy for repair of either isolated pulmonic stenosis, ventricular septal defect or tetralogy of Fallot have been studied.

All cases which showed postoperative prolongation of the QRS complex with right ventricular conduction delay were included in this study. Of the 13 patients who showed prolongation of the QRS complex, 9 patients demonstrated a distinct delay in the onset of contraction of the right ventricle of

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at least 0.02 seconds when compared to the preoperative state. The range of delay in these patients thus far studied has been from 0.02 seconds to 0.06 seconds. In 4 patients with the electrocardiographic configuration of right bundle branch block no delay in right ventricular contraction occurred.

Proposed Course of Project: Continued study of the preoperative and postoperative catheterization records of patients having undergone right ventriculotomy with subsequent development of prolongation of QRS complex, in order to better clarify whether this is true right bundle branch block or parietal block. Correlation with the presence of an open or closed ventricular septal defect at the time of postoperative study will be carried out. Preliminary observations suggest that when right ventricular contraction is delayed the defect is frequently open.

Part B included No

Serial No. NHI-277
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Assessment of the Accuracy of the Kr^{85} Inhalation Test in Detecting Left-to-Right Shunts

Principal Investigator: Allan Goldblatt, M. D.

Other Investigator: Eugene Braunwald, M. D.

Man Years (calendar year 1960):
Total: 0.4
Professional: 0.1
Other: 0.3

Project Description: A total of 380 patients during the past 2½ years have had Kr^{85} inhalation tests during the course of diagnostic cardiac evaluation in an attempt to detect the presence of a left-to-right shunt. In an effort to determine the validity and sensitivity of this test and its comparison to qualitative indicator dilution curves utilizing cardio-green dye, a retrospective study of these patients has been undertaken. Final proof of the existence of a left-to-right shunt either at the pulmonary arterial, right ventricular, or right atrial level, was determined either at operation, post-mortem examination or by use of selective angiocardiography. To date, 77 patient's charts have been reviewed. In 44 of these patients who were subsequently proven to have a left-to-right shunt, the Kr^{85} inhalation test was positive, that is, the result was greater than 15% in the area of the shunt. The qualitative diagnostic dye dilution curves utilizing cardio-green dye, in all patients with proven shunts, were also able to detect the existence of the left-to-right shunt. These dye dilution curves, in addition, were able to detect the existence of a right-to-left shunt which existed concomitantly in many patients.

Proposed Course of Project: It is planned to continue the evaluation of the remaining 303 patients in hopes of drawing more specific conclusions regarding the comparative results of Kr^{85} inhalation tests vs. qualitative indicator dilution curves in detecting the presence of left-to-right shunts.

Part B included Yes

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Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Braunwald, E., Morrow, A. G., Sanders, R. J., and Long, R. T. L.: Characterization of circulatory shunts by foreign gas technics, Congenital Heart Disease, American Association for the Advancement of Science, Washington, D. C., p. 241, 1960.

Long, R. T. L., Braunwald, E., and Morrow, A. G. Intracardiac injection of radioactive krypton. Clinical applications of new methods for characterization of circulatory shunts, Circulation 21:1126, 1960.

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Serial No. NHI-278
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of Cardiac Muscle

Principal Investigators: Maurice M. S. Aygen, M. D.
Eugene Braunwald, M.D.

Other Investigators: None

Man Years (calendar year 1960):
Total: 0.3
Professional: 0.2
Other: 0.1

Project Description: In order to study the static and dynamic properties of cardiac muscle, the following groups of experiments were designed.

1) The relation of fiber length and contractile force of human and dog's hearts: For these experiments the strain gauge arch, capable of measuring the isometric contractile force was employed. The two legs of the instrument were fixed with silk sutures over the muscle segment to be studied. A micrometer screw increased the distance between the two legs by a half millimeter with each turn, so that the muscle segment could be stretched by desirable increments. The observations were made on the right ventricle of seven and on the left ventricle of two patients undergoing cardiac surgery. In all experiments each increment of stretch was accompanied by an increase of resting tension (T_o) and a greater increase of maximal tension (T_m). This obviously resulted in augmentation of active tension (ΔT). At greater extensions the initial tension rose steeper than the maximal tension causing a decline in active tension. The diagrams obtained by plotting the segment length against active tension, were typical of "Starling" curves. These results are the most direct and conclusive evidence of the operation of Starling's law in the human heart. In addition, we found that with increase in contractile force the rate of tension rise became greater. A linear relationship was obtained between segment length at various degrees of stretch and the mean velocity of tension development. $\left(\frac{\Delta T}{t_u} = \frac{\text{active tension}}{\text{upstroke time}}\right)$

These observations showed that cardiac muscle responds to increased strain $\left(\frac{\Delta l}{l_o}\right)$ with an increase in the velocity of the development of tension.

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Individual Project Report
Calendar Year 1960

In the course of this work observations were made on such features as hysteresis loops of length tension diagrams, the stress relaxation, and the magnitude of shortening of muscle segment during systole. The width of hysteresis loops were related to the magnitude of stretch. After a series of repetitive stretches, stable hysteresis loops were obtained. The length tension diagrams of the dog's heart showed the same characteristics as the human hearts.

2) The effect of the heart rate on the contractile force of the cardiac muscle: The fiber length is an important, but not the sole factor, which influences the contractile force of cardiac muscle. Another property of cardiac muscle discovered by Bowdich and confirmed by others is the staircase phenomenon. In order to study this phenomenon the adjustable strain gauge arch was attached to the right ventricle of an anesthetized dog's heart. The muscle segment under study was physiologically dissociated from the remaining cardiac muscle by stretching it to 140% of its original length. It was assumed that by doing so the volume changes of the right ventricle during the experiment will not affect the muscle segment under study. The electrode of a cardiac pacemaker was attached to the appendage of right atrium, and the heart was driven at various frequencies. In each instance when the frequency of electric stimulus was increased the active tension dropped at first and then gradually increased to a higher level than that corresponding to the slow rate. There was also a small rise of initial tension. Sudden slowing of the heart rate produced the opposite changes. That is, marked increase of active tension with the first beat and gradual drop to lower levels in subsequent beats. The arterial pulse pressure changes indicated comparable changes in the left ventricular performance. It was interesting to note that the rise of active tension with higher frequencies was always associated with an increase in velocity of tension development.

3) Post-mortem volume-pressure relationship and length-tension diagrams of human and dog's hearts: The mitral and aortic valves of post-mortem human hearts were sewn together and two balloon catheters were placed in the left ventricle. A series of volume increments were injected at regular intervals through one catheter and continuous pressure recordings were made through the second catheter. The compliance of the left ventricle, the stress relaxation, the volume and segment length relationships were studied.

Length tension diagrams of cardiac muscle were obtained at various intervals after death and they were compared with the resting tension diagrams of living cardiac muscle. Both showed the same sigmoid configuration but the former was displaced to the left.

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Individual Project Report
Calendar Year 1960

4) The effect of Levophed on the length-tension diagrams of mammalian cardiac muscle: These observations are too few and incomplete to derive conclusions.

Proposed Course of Project: 1) by employing specially designed needles or other arrangements it will be possible to study geometrical segments of the cardiac muscle with measurable diameters. This will allow to make comparable quantitative studies, and 2) to study the effect of levophed on the dynamic properties of cardiac muscle.

Part B included No

Serial No. NHI-279
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Serum Glutamic Oxaloacetic Transaminase Levels After Cardiac Surgery

Principal Investigator: Charles J. Frahm, M. D.

Other Investigators: None

Man Years (calendar year 1960):
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description: There have been few reports on the effects of cardiac surgery on serum glutamic oxaloacetic transaminase levels. Since the transaminase level is an important diagnostic test in many patients for determining whether or not a myocardial infarction has occurred, it is important to know what levels are obtained after surgery which traumatizes heart muscle. Thus far 134 patients have been studied. There have been 30 patients with atrial septal defects, 22 with mitral stenosis, 20 with aortic stenosis, 13 with patent ductus, 10 with ventricular septal defects, 8 with tetralogy of Fallot, 7 with pulmonic stenosis, etc.

The control levels have ranged from 13 - 36 units per cent and the elevated levels have ranged from 42 - 363 units per cent. All patients had elevated levels after operation. There is a good correlation between the degree of elevation and the amount of heart muscle incised. The maximum elevations occur during the 1st 24 - 48 hours after surgery. These data indicate that elevations of the serum glutamic oxaloacetic transaminase occur immediately following cardiac operations and that such elevations cannot be used to detect the presence of myocardial infarction in the early postoperative period.

Proposed Course of Project: Completed

Part B included No

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Total. 0.2
Preferences. 0.2
Other. 0.1
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Serial No. NHI-280
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Influence of Triiodothyronine on Digitalis Requirements

Principal Investigators: Robert L. Frye, M. D.
Eugene Braunwald, M. D.

Other Investigators: None

Man Years (calendar year 1960):
Total: 0.4
Professional: 0.3
Other: 0.1

Project Description: The effects of triiodothyronine (T_3) administration on digitalis requirements was studied in a group of patients with atrial fibrillation. The ventricular rate was utilized to provide a quantitative expression of digitalis effect. In one patient the amount of acetylstrophanthidin, administered as a constant infusion, required to slow the ventricular rate to 70 beats/minute rose from an average of 0.58 mg. while he was myxedematous, to 1.50 mg. when he was euthyroid. When mild thyrotoxicosis was induced in three euthyroid patients the daily dose of digoxin had to be increased approximately four-fold in order to maintain the ventricular rate at a control level. In three patients it was observed that reserpine or syrosingopine administered intramuscularly abolished the increased requirements for digoxin induced by T_3. In two patients syrosingopine was found to partially prevent the tachycardia resulting from large doses of intravenous T_3. The possible mechanisms responsible for these effects and their therapeutic implications are discussed.

Proposed Course of Project: It is planned to evaluate the effect of triiodothyronine on the inotropic action of digitalis in dogs using the Walton-Brodie strain gauge arch. It is proposed to study the effect of a given dose of acetylstrophanthidin on myocardial contractile force when the dogs are myxedematous, after they have been rendered euthyroid and finally after they have been rendered hyperthyroid.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Frye, R. L., and Braunwald, E.: Studies on Digitalis III. The influence of triiodothyronine on Digitalis requirements, Circulation. In press.

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Serial No. NHI-281
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Role of Carotid and Intracardiac Baroreceptors and of Vasoactive Drugs in Control of the Venous Bed

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: Charles J. Frahm, M. D.
Eugene Braunwald, M. D.

Man Years (calendar year 1960):
Total: 0.7
Professional: 0.4
Other: 0.3

Project Description: The effects of stimulation of carotid and intracardiac baroreceptors on the capacity of the total systemic vascular bed, on venous distensibility and venous return were studied in an experimental preparation in which the use of extracorporeal circulation permitted complete hemodynamic isolation of the carotid sinuses and of the heart and lungs. Alterations of intravascular blood volume and venous return were measured continuously. Brief occlusion of the venous outflow line permitted inscription of venous pressure-volume curves. Elevation of perfusion pressure in the isolated carotid sinuses (4 dogs) produced venodilatation and an augmentation of intravascular blood volume, averaging 12.9 ml./Kg.; when carotid sinus pressure was decreased, venoconstriction occurred, intravascular blood volume diminished and venous return to the oxygenator increased by 27 per cent of the control level. Elevation of intracardiac pressures (8 dogs) produced venodilation with an increase of intravascular blood volume, averaging 6.9 ml./Kg., and a decrease in venous return, averaging 25 per cent of control perfusion rates. Infusion of norepinephrine (7 dogs) resulted in venoconstriction with a decline in intravascular volume averaging 19.0 ml./Kg., and an increase in venous return, averaging 40 per cent of the control level. Arfonad infusion (4 dogs) resulted in venodilatation, an augmentation of blood volume averaging 17.3 ml./Kg., and a decrease in venous return averaging 30 per cent of the control. Alterations in venous distensibility were consistent with the observed changes in vascular capacity. These experiments indicate that the distensibility of the total venous bed, the total intravascular capacity, and venous return are controlled reflexly by baroreceptors in the carotid sinuses and heart and are modified directly by vasoactive drugs.

Proposed Course of Project: Completed

Part B included Yes

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PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Ross, J., Jr., Frahm, C. J., and Braunwald, E.: The influence of the carotid baroreceptors and of vasoactive drugs on vascular capacity and venous distensibility, Circulation Res. In press. (Jan. 1961)

Ross, J., Jr., Frahm, C. J., and Braunwald, E.: The influence of intracardiac baroreceptors on venous return, systemic vascular volume and peripheral resistance, J. Clin. Invest. In press.

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Serial No. NHI-282
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Thermal Dilution Curves in the Study of Circulatory Shunts

Principal Investigator: Theodore Cooper, M. D., Ph.D.

Other Investigators: Eugene Braunwald, M. D.
Grant C. Riggle
Andrew G. Morrow, M. D.

Man Years (calendar year 1960):
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description: Indicator dilution curves constitute one of the most useful technics in the recognition and localization of circulatory shunts. The necessity for the withdrawal of significant quantities of blood and the distortion in the dilution curve imposed by the sampling system have prompted the development of technics permitting introduction of the detecting element directly into the blood stream. The feasibility of recording intravascular dilution curves, utilizing a thermal sensitive element as the detector and solutions of cold saline as the indicator, has been demonstrated in the experimental animal. A transistorized DC amplifier, which when used with a thermistor permits the recording of stable linear thermal dilution curves has been developed. The applicability of the thermal dilution curves for the study of patients with circulatory shunts, both in the Cardiac Catheterization Laboratory and in the operating room has been demonstrated.

Proposed Course of Project: Project completed. The technique is currently employed routinely in diagnosis in our Catheterization Laboratory.

Part B included Yes

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PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Cooper, T., Braunwald, E., Riggle, G. C., and Morrow, A. G.: Thermal dilution curves in the study of circulatory shunts. Instrumentation and clinical applications, Am. J. Cardiol. In press.

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Serial No. NHI-283
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Study of Patients with Idiopathic Hypertrophic Subaortic Stenosis

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: Andrew G. Morrow, M. D.
William P. Cornell, M. D.
Maurice M. S. Aygen, M. D.
Theodore F. Hilbish, M. D.

Cooperating Units: Diagnostic X-ray Department, Clinical Center

Man Years (calendar year 1960):
Total: 0.6
Professional: 0.4
Other: 0.2

Project Description: This project consists of a clinical and hemodynamic description of 15 patients with idiopathic hypertrophic subaortic stenosis who have been studied at the National Heart Institute.

The diagnosis of idiopathic hypertrophic subaortic stenosis, i.e. left ventricular hypertrophy producing severe obstruction to left ventricular outflow has been established in 15 patients. All had systolic murmurs, most prominent either at the mitral or tricuspid areas. Left ventricular systole was prolonged, resulting in paradoxical splitting of the second heart sound. Electrocardiograms showed either left ventricular hypertrophy or anomalous atrioventricular excitation, and roentgenograms demonstrated left atrial and ventricular enlargement without aortic dilatation. Left heart catheterization revealed systolic pressure gradients within the ventricle ranging from 40 to 185 mm.Hg, and localized the site of obstruction to the left ventricular outflow tract in every patient. The left ventricular pressure pulses exhibited a characteristic notch in early ventricular systole, and a striking rise during atrial contraction. Both the palpable and recorded peripheral arterial pressure pulses rose rapidly during early systole, unlike those in valvular aortic stenosis. Left ventricular angiocardiogram demonstrated a markedly thickened ventricular wall obstructing the outflow tract of the left ventricle only during a portion of the cardiac cycle. At open operation in five patients the angiocardiographic interpretations were confirmed; in two patients the hypertrophied muscular ring was incised longitudinally to relieve the obstruction and the early clinical and hemodynamic results of this operation are encouraging. With the delineation of its clinical, hemodynamic, angiocardiographic and anatomic features, idiopathic hypertrophic subaortic stenosis

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emerges as a specific entity which can be distinguished preoperatively from discrete valvular and subvalvular aortic stenosis. Its recognition is important in selecting for operation patients with all forms of obstruction to left ventricular outflow and in planning the surgical procedure.

Proposed Course of Project: To follow these and other patients with idiopathic hypertrophic subaortic stenosis in order to learn the actual history of the disease and specifically to evaluate the effects of the operation on this malformation.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Articles in Periodicals:

Braunwald, E., Morrow, A. G., Cornell, W. P., Aygen, M. S., and Hilbish, T. F.: Idiopathic hypertrophic subaortic stenosis: Clinical, hemodynamic and angiographic manifestations, Am. J. Med. In press. (December 1960).

Braunwald, E., Morrow, A. G., and Cornell, W. P. (introduced by R. W. Berliner): Idiopathic hypertrophic subaortic stenosis: Anatomic, hemodynamic, clinical and angiocardiographic factors, Trans. Assoc. Am. Phys. 1960.

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Serial No. NHI-284
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Observations in Man on the Effect of Digitalis on the Contractility of the Non-Failing Heart and on Total Vascular Resistance

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: Robert D. Bloodwell, M. D.
Lëon I. Goldberg, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1960):
Total: 0.6
Professional: 0.4
Other: 0.2

Project Description: The direct effects of two digitalis preparations on the contractile force of the heart were studied by means of the Walton-Brodie strain gauge arch in 21 patients who had never experienced congestive heart failure. Fourteen patients with either atrial septal defect or pulmonic stenosis studied during cardiopulmonary bypass were given 0.5 to 1.5 mg. of acetylstrophanthidin, a dose which averaged 0.026 mg./Kg. body weight. This resulted in an increase in contractile force which ranged from 16% to 327% and averaged 89% of the contractile force measurement prior to digitalization. In three patients who received an average dose of 0.022 mg./Kg. of Lanatoside C during cardiopulmonary bypass, the right ventricular contractile force increased by an average of 31% of control levels. In an attempt to study the response of a ventricle which had never been subjected to abnormal hemodynamic burdens, left ventricular contractile force was recorded immediately after mitral valvulotomy in three patients. Acetylstrophanthidin increased contractile force an average of 24% of the control levels. The administration of the digitalis preparations to patients on cardiopulmonary bypass at a constant perfusion rate permitted study of the direct effects of these drugs on systemic vascular resistance. Acetylstrophanthidin resulted in a brief increase in systemic vascular resistance which averaged 23% and Lanatoside C resulted in an average increase of 14% of the control levels. It is concluded that these digitalis preparations augment the contractile force of the non-failing human heart and constrict the systemic vascular bed.

Proposed Course of Project: It is planned to ascertain whether the responsiveness of the myocardium to digitalis is modified by the failure state or whether the inotropic effect which is seen in the non-failing heart

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also exists in the failing heart. To this end we plan to study the contractile force response to acetylstrophanthidin in dogs without heart failure and after right ventricular failure has been produced by means of pulmonic stenosis and tricuspid regurgitation.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Braunwald, E., Bloodwell, R. D., Goldberg, L. I., and Morrow, A. G.: Studies on Digitalis IV. Observations in man on the effects of Digitalis preparations on the contractility of the non-failing heart and on total vascular resistance, J. Clin. Invest. In press. (January 1961).

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Serial No. NHI-285

1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Applicability of Starling's Law of the Heart to the Circulation in Intact Man

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: Charles J. Frahm, M. D.
John Ross, Jr., M. D.

Man Years (calendar year 1960):
Total: 0.5
Professional: 0.2
Other: 0.3

Project Description: The applicability of Starling's law of the heart to the circulation of the intact man has been studied in seven subjects. Left ventricular filling pressure has been measured by means of transseptal left heart catheterization and simultaneous esophageal balloon pressure. Cardiac output was measured by means of the indicator-dilution technique. The autonomic nervous system of the subjects was blocked by means of a continuous infusion of Arfonad and the response of ventricular filling pressure and cardiac output to the infusion of 1500 cc. of blood was evaluated. In all subjects studied so far, a consistent linear relationship between left ventricular stroke work and left ventricular filling pressure has been noted, thus supporting the concept that Starling's law of the heart applies to circulation of intact man after ganglionic blockade has been established.

Proposed Course of Project: This project has been completed. The data are being evaluated and they are being readied for early publication.

Part B included No

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Serial No. NHI-286
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Hemodynamic Observations on the Function of the Left Atrium in Man

Principal Investigator: Eugene Braunwald, M. D.

Other Investigator: Charles J. Frahm, M. D.

Man Years (calendar year 1960):
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description: It has recently been emphasized by Sarnoff, Mitchell, and Gilmore that atrial contraction contributes to ventricular filling, but the clinical importance of this phenomenon has not been appreciated. The difference between left ventricular end-diastolic or left atrial "z" point pressure (LVEDP) and mean left atrial pressure (MLAP) provides a quantitative measure of left atrial function. This pressure difference expresses the ability of the atrium to contribute to left ventricular filling, to augment LVEDP, and thus to provide the stimulus for ventricular contraction while maintaining MLAP, and therefore pulmonary venous pressure, at a lower level. In 9 subjects without heart disease, and in 7 patients with hemodynamically trivial abnormalities, LVEDP-MLAP ranged from -4 to +3 and averaged 0 mm.Hg. When MLAP was elevated to an average of 19 mm.Hg in 6 subjects by means of rapid blood transfusion, LVEDP-MLAP remained low, averaging 0 mm.Hg. In 23 patients with left ventricular hypertrophy due to aortic valve disease, idiopathic myocardial hypertrophy, or coronary artery disease, the LVEDP averaged 21 mm.Hg; the LVEDP-MLAP averaged 6 mm.Hg and was less than 3 mm.Hg, the highest normal value, in only 2 patients. An atrial sound was easily audible in the majority of these patients.

These data emphasize the clinical importance of left atrial contraction in patients with left ventricular hypertrophy in whom left atrial contraction elevated LVEDP while maintaining MLAP and pulmonary venous pressure at a significantly lower level. These observations provide an explanation for the deleterious effects of atrial arrhythmias in patients with left ventricular hypertrophy.

Proposed Course of Project: This project has been completed and is being written up for early publication.

Part B included No

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Serial No. NHI-287
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Observations in Man and the Beat-to-Beat Relationship Between Left Ventricular Segment Length, Filling Pressure and the Characteristics of Ventricular Contraction

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: Robert L. Frye, M. D.
Maurice M. S. Aygen, M. D.
Joseph W. Gilbert, Jr., M. D.

Man Years (calendar year 1960):
Total: 0.7
Professional: 0.4
Other: 0.3

Project Description: The present investigation was designed to determine whether left ventricular end-diastolic segment length and end-diastolic pressure are important determinants of ventricular contraction. Systemic arterial pressure and effective end-diastolic pressure were measured at operation in 25 adult patients with rheumatic mitral valve disease and atrial fibrillation. Continuous alterations of the length of a segment of left ventricular muscle were recorded simultaneously in 12 of these patients by means of a mercury-filled resistance gauge sutured to the surface of the left ventricle. The variability of the duration of diastole resulted in beat-to-beat alterations of ventricular filling which resulted in variations of end-diastolic segment length and pressure. For each individual beat the peak systolic ventricular pressure was utilized as an index of maximum tension developed; the area beneath the left ventricular pressure curve (Tension-Time Index) was employed as a measure of the total tension developed; the systolic period indicated the duration of the tension state; the systemic arterial pulse pressure was utilized to indicate relative changes in stroke volume. In every patient alterations in end-diastolic segment length correlated closely with all four parameters utilized to characterize ventricular function. In order to determine whether these relationships also exist in intact unanesthetized subjects, left ventricular end-diastolic pressure was determined by means of transseptal left heart catheterization in four patients. The correlations of end-diastolic pressure with systolic pressure, tension-time index, duration of systole and arterial pulse pressure were similar to those obtained in the patients studied at operation. These results are consistent with the view that Starling's law of the heart operates in man on a

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Calendar Year 1960

beat-to-beat basis and that end-diastolic segment length and end-diastolic pressure are important determinants of the characteristics of the subsequent ventricular contraction.

Proposed Course of Project: It is planned to determine whether the relationship between end-diastolic segment length and the characteristics of the subsequent contraction can be modified by the infusion of drugs such as nor-adrenalin and digitalis in the course of operation.

Part B included Yes

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Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Braunwald, E., Frye, R. L., Aygen, M. S., and Gilbert, J. W., Jr.: Studies on Starling's law of the heart. III. Observations in patients with mitral stenosis and atrial fibrillation on the relationships between left ventricular end-diastolic segment length, filling pressure, and the characteristics of ventricular contraction, J. Clin. Invest. In press. (December 1960).

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Serial No. NHI-288
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Use of Solutions of Kr^{85} for the Measurement of Cardiac Output

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: William P. Cornell, M. D.
Edwin C. Brockenbrough, M. D.

Man Years (calendar year 1960):
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description: Solutions of Kr^{85} may be employed as the indicator for the measurement of cardiac output by the indicator-dilution technic. This can be accomplished by injecting Kr^{85} into the left side of the heart and sampling from a systemic artery or by injecting Kr^{85} into a peripheral vein and sampling from the pulmonary artery. The advantage of this technic is based on the fact that the krypton does not recirculate and the entire dilution curve need not be inscribed. Thus, a blood sample is obtained at a constant rate from either the pulmonary artery or the femoral artery following injection of Kr^{85}. The analysis of this sample for Kr^{85} content provides enough information to calculate the cardiac output, provided the time of sampling and the amount of Kr^{85} injected are both normal. The accuracy of this technic has been tested in three systems. 1) In a simple circulatory model the Kr^{85} outputs were checked against timed collections of the flow, 2) Secondly, in dogs in which a rotameter was used to measure cardiac output, the accuracy of the Kr^{85} method has been proved, and 3) Comparisons of the Kr^{85} outputs have been made in humans at the time of left heart catheterization with the cardiac outputs obtained by means of the standard cardio-green indicator dilution methods. In all three of these systems the Kr^{85} outputs corresponded closely with the simultaneously determined flows. The advantages of this technic are that it avoids the use of a densitometer and greatly simplifies both the performance of the cardiac output, the necessary instrumentation and the calculations.

Proposed Course of Project: The project has been completed and the data is being prepared for publication.

Part B included No

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Serial No. NHI-289

1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Simplified Technique for the Detection of Patent Ductus Arteriosus

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: William P. Cornell, M. D.

Man Years (calendar year 1960):
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description: A simplified technique for the detection of left-to-right shunts originating from the aorta was developed. A thin, radiopaque catheter is introduced into the aortic arch through a Cournand needle placed in the femoral artery, and injections of a solution of Kr^{85} are made. In the presence of a shunt, the injected Kr^{85} is rapidly detected in the expired air, but in the absence of a shunt the appearance time of Kr^{85} in the expired air is delayed. When an aortic shunt is present it may be localized by means of injections of cardio-green dye at various points in the aorta by sampling the blood from the right brachial artery. The clinical value and reliability of these techniques was demonstrated in a series of patients with various forms of congenital or acquired heart disease. It is of considerable value in determining the presence or absence of a patent ductus arteriosus in patients with a ventricular septal defect prior to open heart operation.

Proposed Course of Project: This project has been completed, and the technique is in current use routinely in our catheterization laboratory.

Part B included Yes

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PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Braunwald, E., and Cornell, W. P.: A simplified technique for the detection of patent ductus arteriosus and of other left-to-right shunts originating from the aorta, Circulation. In press.

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Serial No. NHI-290
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Evaluation of Transseptal Left Heart Catheterization and the Extensions of this Technique to Left Heart Angiocardiography.

Principal Investigator: Edwin C. Brockenbrough, M. D.
Eugene Braunwald, M. D.

Other Investigators: John Ross, Jr., M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1960):
Total: 0.8
Professional: 0.5
Other: 0.3

Project Description: The technique of transseptal left heart catheterization which was developed in this laboratory two years ago is being evaluated continuously. More than 300 procedures have now been carried out without any mortality and only minimal morbidity. A modified transseptal needle was developed for left atrial angiocardiography. Efforts have also been directed toward extending the usefulness of transseptal left heart catheterization by modifying the procedure so as to permit selective left ventricular angiocardiography. This was accomplished by use of the recently developed Seldinger technique. By this technique the femoral vein is punctured by means of a needle; a stylet is introduced, the needle is removed and a special catheter is introduced along the stylet into the right side of the heart. A small transseptal needle then punctures the atrial septum and the catheter is then advanced through the atrial septum into the left atrium across the mitral valve and into the left ventricle. This technique permits left ventricular angiocardiography and permits both right and left heart catheterizations to be carried out without requiring surgical exposure of the saphenous vein. This technique has now been used successfully, without any difficulty, in 25 patients. Left ventricular angiocardiograms, and in several instances, left atrial angiocardiograms have also been performed without any difficulty.

Proposed Course of Project: Further refinement of the technique is possible and current efforts are being directed toward development of more suitable catheters.

Part B included Yes

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Individual Project Report
Calendar Year 1960

Part B

Articles in Periodicals:

Ross, J., Jr., Braunwald, E., and Morrow, A. G.: Transseptal left heart catheterization: A new diagnostic method, Progr. Cardiovasc. Dis. 2:315, 1960.

Ross, J., Jr., Braunwald, E., and Morrow, A. G.: Left heart catheterization by the transseptal route: A description of the technique and its applications, Circulation In press.

Brockenbrough, E. C., and Braunwald, E.: A new technic for left ventricular angiocardiography and transseptal left heart catheterization, Am. J. Cardiol. In press.

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Serial No. NHI-291
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Hemodynamic Technique for the Detection of Idiopathic Hypertrophic Subaortic Stenosis

Principal Investigator: Edwin C. Brockenbrough, M. D.

Other Investigator: Eugene Braunwald, M. D.

Man Years (calendar year 1960):
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description: In the presence of valvular aortic stenosis or of discrete subvalvular stenosis, the narrowed orifice is constant and its size is not altered by changes in the force of ventricular contraction. In hypertrophic subaortic stenosis the orifice narrows during systolic contraction of the hypertrophied muscle in the left ventricular outflow tract and the orifice size therefore is a function of the force of left ventricular contraction. In 75 patients, proved to have the discrete, "fixed" type of aortic or subaortic stenosis the beats following the compensatory pause after a premature contraction were always characterized by higher left ventricular systolic pressures and larger systemic arterial pulse pressures than the normal beats, i.e. arterial pulse pressure varied directly with left ventricular systolic pressure and with the duration of diastole. In 12 patients proved to have hypertrophic subaortic stenosis, the beats following premature contractions always exhibited lower arterial pulse pressures than did the normal beats, i.e. arterial pulse pressure varied inversely with left ventricular systolic pressure and with the duration of diastole. The distinction between these two types of obstruction to left ventricular outflow may be established with confidence by this analysis of the effects of premature ventricular contractions on either the simultaneously recorded left ventricular and arterial pressure pulses, or on the arterial pressure pulses alone. The recognition of hypertrophic subaortic stenosis is essential in formulating a rational plan of treatment for all patients with obstruction to left ventricular outflow, and the hemodynamic technique described affords a simple but reliable diagnostic approach.

Part B included Yes

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PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Article in Periodical:

Brockenbrough, E. C., Braunwald, E., and Morrow, A. G.: A hemodynamic technique for the detection of idiopathic hypertrophic subaortic stenosis, Circulation. In press.

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Serial No. NHI-292
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Development of Nuclear Magnetic Apparatus for Blood Flow Measurement

Principal Investigators: Vsevolod Kudravcev
Robert L. Bowman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960) Patient Days: None
Total: 1.15
Professional: 1.15

Project Description:

Progress During Past Year:

During the past year, the previously constructed NMR devices were applied to experimental investigation at the best method for flow signal measurement. The method chosen for a systematic investigation was the absorption of R. F. energy by protons in a liquid containing hydrogen when subjected to a strong D. C. magnetic and perpendicular R. F. field of appropriate frequency. The basic advantage of the method is the possibility to perform very rapid measurements when the flow rate varies with time. The flow variation can be measured as the signal amplitude variation, and a calibration of flow curves can be obtained directly by simple means. The experimental apparatus has been modified to obtain optimal results in the absorption method. Also considerable time was spent on refinement in the needed electronic circuitry for improved reproducibility. The improved reproducibility coupled with simplicity of initial adjustment have been achieved by development of a new detection circuit. The circuit uses a self limited oscillator with a quartz-filtered feedback loop. This modification allows a reduction in the number of vacuum tubes required, and permits improved signal to noise ratio, due to straight and balanced R. F. and audio amplification. One new experimental flowmeter was built around the above circuit. This made substantial progress toward the goal of utilizing NMR to measure the flow of blood and other biological fluid in "situ".

We feel that the new circuit, after some necessary improvements in the input, solves the electronic problems involved in an NMR flowmeter.

The second part of the problem is the magnetic field source. The magnet unit still needs considerable development towards miniaturization, to achieve a practical size flow-detection head useful for biological applications. The development of a practical magnet unit, by utilization of pulse technique, is under preliminary investigation.

The above outlined electronic circuit was utilized for continuous flow measurement of blood, saline, and water, in combination with the present magnet unit and showed satisfactory flow response.

Experimental conditions were arranged so that the liquid temperature can be raised 20° above room temperature and the results recorded. The experiments show a negligible shift at initial calibration with increased temperature up to 35°.

In one system, injection into the flow line is possible. Liquids containing paramagnetic impurities possessed different relaxation time (T_1). This effect was investigated for the purpose of developing trace and dilution techniques for flow measurement. NMR absorption responses for liquids of different paramagnetic concentrations were studied. Also, through systematic experiments with different flow-probe tubes, we accumulated data on optimal R. F. level and probe geometry, in order to obtain an optimal absorption signal in liquids of various relaxation time magnitudes. For recording the flow curves, a two-channel magnetic recording system has been set up, and a switching and inter-connection system designed. Also, a transistorized playback amplifier was designed and constructed. We hope the system can be very useful for future investigation of pulsatile flow and for recording blood flow in the circulatory system of experimental animals. The new electronic circuit, due to its wide range R. F. output, can be utilized also for relaxation time measurements (progressive saturation method) on different biological substances for analytical or diagnostic purposes. The exact determination of relaxation times is of great importance in the flow measurement technique, because the NMR flow measurement technique employs the modification of T_1 by flow.

The best method of relaxation time measurement can be achieved by use of the spin echo technique. The spin echo technique was investigated at the Spin Echo NMR Workshop at Schlumberger Corporation in January, 1960. During the workshop, the relaxation time of blood was measured and various other data obtained. Spin echo apparatus was designed and constructed in our laboratory for precise relaxation time measurement by several spin echo methods. Although requiring more elaborate electronics, these have potential possibilities of precise and simple measurement by utilization of

a small pulse type solenoid as the source of magnetic field.

Direction of Current Research:

We hope to utilize the pulse and spin echo techniques for study of flow, as well as analytical purposes, after a more complete investigation of the absorption method now employed.

Preliminary experiments on the use of NMR absorption for determining the distribution of hydrogen and some other NMR susceptible nuclei in living tissue have yielded interesting results. This work will be continued in the hope that a scanning technique will give coarse pictures (in intensity variation) of muscle, arteries and other structures. It also may be possible to map the distribution of injected NMR susceptible nuclei. NMR susceptible nuclei are listed in the following chart:

Isotope
Hydrogen - H_1, H_2, H_3
Fluorine - F_{19}
Phosphorus - P31
Nitrogen - N14
B11, C13, O17, Si29, Li17, Na23, AL27, Mg 25, S23, CL35, K39, Cu63,Cu65

Part B included - Yes

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PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Kudravcev, Vsevolod and Bowman, Robert L.: "Utilization of Nuclear Magnetic Resonance for Flow Rate Measurement", Digest of Papers, 13th Annual Conference on Electrical Techniques in Medicine and Biology, p. 21, October 21, November 1 & 2, 1960, Washington, D. C.

Serial No. NHI-293
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Analog Computer for Analysis of Sums of Exponentials

Principal Investigator: Frank W. Noble

Other Investigators: J. P. Dustin

Cooperating Units: Laboratory of Cellular Physiology and Metabolism, National Heart Institute.

Man Years (calendar year 1960)
Total: 0.1
Professional 0.1

Project description:

Progress During Past Year:

Studies of the life span of radioactive tracers in the blood involve the analysis of a curve consisting of the sum of two or more exponential functions into the component exponentials. While this analysis may be done by a graphical process, the procedure is tedious. It seemed possible that a special analog computer might be employed to speed the analysis and possibly improve on the accuracy attainable.

A computer has been built which synthesizes exponential functions at a repetition frequency of 60 cps. The initial value and the exponent of each of the functions can be varied independently. The sum is presented on a projection oscilloscope and compared with the data curve. Adjustments are made to obtain the best fit, after which the component exponentials are read out individually.

Direction of Current Research:

Actual data curves have been produced in proper form for analysis by the computer. Comparisons of the components found by the use of the computer with the components found by graphical means will be made.

Part B included - No

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Serial No. NHI-294
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Hydraulic Pressure Generator

Principal Investigator: Frank W. Noble

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Progress During Past Year:

In order to determine the dynamic performance of cardiac manometers it is necessary to have a generator of hydraulic pressure waves of known intensity, frequency, and contour. The object of this project is to produce such a generator.

A new model of the driving amplifier was built and tested. This unit is currently being used in the compensation of the dynamic response of cardiac catheters (see report "Catheter Compensating Amplifier" by F. Noble).

Direction of Current Research:

A final design for the electronic driver amplifier and square wave generator is in process. This equipment will incorporate the oscillator, power supply, and power amplifier in a single small unit so that it will be more convenient to use.

Part B included - yes

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PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Noble, Frank W., "A Hydraulic Pressure Generator For Testing The Dynamic Characteristics of Blood Pressure Manometers", The Journal of Laboratory and Clinical Medicine, St. Louis., Vol. 54, No. 6, Pages 897-902, December, 1949.

Serial No. NHI-295
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Gas Chromatograph Detector Based on Measurement of Sound Velocity

Principal Investigator: Frank W. Noble

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1960)
Total: 0.6
Professional: 0.5
Other: 0.1

Patient Days: None

Project Description:

Progress During Past Year:

The density of the effluent from a gas chromatographic column varies when components of the sample are emerging. Since the velocity of sound is affected by the density of the medium through which it travels, a device which measures the sound velocity can be used as a detector for gas chromatography.

For ideal gases it can be shown that if a minute amount of a foreign substance having a molecular weight much larger than the carrier gas appears in the effluent, the phase change will be

$$\phi = \frac{180}{M_1} \frac{s}{V_1} \frac{f}{} a (M_2 - M_1)$$

where: f = sound frequency
s = sound path length
a = mole fraction of sample gas 2
V_1 = sound velocity in carrier gas 1
M_1 = molecular weight of carrier gas 1
M_2 = molecular weight of sample gas 2

The above equation will be correct within 10 percent if the molecular weight ratio exceeds 8 and the mole fraction is less than one part in one thousand. If these conditions cannot be met, a somewhat more involved expression must be used.

A better test cell has been designed and put into use. This cell can be operated at temperatures up to 200 degrees centigrade and is readily adaptable to use with different carrier gases. Since the attenuation of the sound path must fall within well-defined limits and since the attenuating properties of different carrier gases vary widely, it is necessary to change the length of the sound path. This is readily accomplished in the new design by simply threading the crystal holders into teflon pipes of differing length.

Considerably better shielding and decoupling have been installed in the single-cell 4 mc unit. This unit has been used to check the agreement between the experimental results and the equation given above.

In these tests, a three foot coil of 1/4 inch copper tubing was installed between the sample injection point and the cell. The function of the tubing was to allow the sample to diffuse, resulting in a record of a skewed Gaussian peak having sufficient width so that its area could be measured with accuracy. The samples were diluted with carrier gas in a 60 cc sample tube, the tube was heated to mix the gases, and the injection was made with a 30 micro liter syringe. For each of the resulting peaks, an appropriate time period was selected and the area under the peak during this period was measured with a planimeter. The mean ordinate, or average phase shift, was then obtained. The volume of carrier gas which flowed through the cell during this period was determined from the flow rate. From the volume of the sample, the average mole fraction was obtained. Substitution of this information into the formula yielded a phase shift which should have been the same as the average phase shift obtained from the experimental curve. The agreement was within 10 percent for a series of five gases having molecular weights progressively increasing from methane to freon 12. In view of the difficulty of obtaining accurate gas mixtures by the method outlined above, it is believed that the tests indicate a confirmation of the equation within the limits of experimental error.

Direction of Current Research:

A sound detector cell having a very small sample volume and high speed for use with capillary columns is under consideration. This cell would have to operate at higher sound frequencies, involving a redesign of the electronic equipment.

The possibility of using sound velocity measurement in liquid chromatographic columns is also promising. It is not possible to use most of the gas detectors in this fashion. We are currently looking into the classical mathematics of transmission in liquids.

Part B included - yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Noble, Frank W.: "Gas Chromatograph Detector Based on Measurement of Ultrasonic Velocity", Digest of Papers, 13th Annual Conference on Electrical Techniques in Medicine and Biology, pp. 12,13. Held October 31, November 1 & 2, 1960, Washington, D. C.

Serial No. NHI-296
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Catheter Compensating Amplifier

Principal Investigator: Frank W. Noble

Other Investigators: Guy Barnett (GMET)

Cooperating Unit: General Medicine and Experimental Therapeutics Branch

Man Years (Calendar year 1960)
Total: 0.3
Professional: 0.2
Other: 0.1

Patient Days: None

Project Description:

Progress During Past Year:

The dynamic performance of cardiac catheters as they are commonly used leaves much to be desired. The amplitude response exhibits a sharp peak of several hundred percent at the first system resonance and the phase response is far from linear. A compensating amplifier having proper amplitude and phase characteristics may be connected between the pressure gauge and the recorder so as to improve the response of the overall system.

A compensating amplifier has been constructed and tested with the Statham P-23 D gauge and several different cardiac catheters. The response of a typical catheter system shows a resonant peak of 385 percent at 48 c.p.s. and very poor phase linearity. When the compensating amplifier is connected, this same system is flat to within 5 percent to 55 c.p.s. and has good phase linearity.

An improved version of the compensating amplifier has been built and partially tested. This model uses a somewhat different circuit in which the gain and d. c. position do not vary as the compensation is changed. These features add greatly to the operational convenience.

Direction of Current Research:

Since there is a correspondence between the sine wave response of a linear system and the square wave response, we are investigating the practicality of making the compensating adjustments while observing the response to periodic square waves of pressure. If this can be done, it will reduce the time required to test out the system.

Part B included - Yes

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PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Noble, Frank W.: "An Electric Circuit for Improving the Dynamic Response of the Conventional Cardiac Catheter System", Digest of Papers, 13th Annual Conference on Electrical Techniques in Medicine and Biology, pp. 82, 83. Held October 31, November 1 & 2, 1960, Washington, D. C.

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Serial No. NHI-297
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Analog Computer for Fourier Analysis

Principal Investigator: Frank W. Noble

Other Investigators: Dali Patel

Cooperating Units: General Medicine and Experimental Therapeutics Branch, National Heart Institute.

Man Years (calendar year 1960)
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Progress During Past Year:

The repetitive analog computer for Fourier Analysis has been described in previous reports. Briefly, the instrument regenerates the desired waveform at high speeds, while simultaneously generating the first six sines and cosines in the Fourier series. The function and the sum of the sinusoidal waves are added and the sum is applied to a square-law voltmeter. The amplitudes of the sine and cosine waves are adjusted one by one to minimize the meter reading. When a minimum is obtained, the coefficients are read out by a switching procedure.

The limit to the accuracy of the process is set by the accuracy with which the function can be regenerated. A much improved model of the function generator has been built and given preliminary tests. This generator is much more stable and accurate than previous attempts.

Direction of Current Research:

The engineering phase of this project is complete. Dr. Patel and others are operating the computer to analyze pressure and flow curves taken from the cardiovascular systems of dogs. The results will be compared with analyses obtained by a digital computer to determine if the accuracy of the analog device is sufficient for their purposes.

Part B included - No

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Serial No. NHI-298

Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Mathematical Investigation of Biological Transport Problems

Principal Investigator: John L. Stephenson

Other Investigators: Arnold Jones, Dr. Donald Fredrickson (LCPM) (LTD)

Cooperating Unit: Dr. Donald Fredrickson, Laboratory of Cellular Physiology and Metabolism

Man Years:
Total 1.5
Professional: .75
Other: .75

Project Description:

Progress During Past Year:

The general purpose of this research has remained to develop a general theory of transport phenomena in linear biological systems and to apply it to particular biological problems. The general theory has been extended in a paper presented at the Fourth Berkeley Symposium on Probability and Statistics. The results are best summarized by quoting from the summary of this paper: ". . . . we represent the state of a biological system by a vector in an abstract space and consider the basic characterization of the system to be a linear operator in the space. We have outlined how the structure of the operator can be obtained from experimental data. It is clear that this representation is primarily a data reduction scheme. Until the method has been applied to a larger number of biological problems, it is difficult to assess its ultimate usefulness. One of the greatest assets of this approach is that it provides a precise mathematical language in which a large number of systems can be described and in terms of which experiments can be formulated. This we have tried to illustrate with the example from fatty acid transport and metabolism. Another great advantage of the method is that both stationary and non-stationary problems can be handled by it, utilizing the same formal language. The method does not yield any direct information about the microscopic structure of a system, but any microscopic theory must yield an operator which agrees with that derived directly from the experimental data, in much the same way that

thermodynamic coefficients computed by statistical theory must agree with those experimentally measured."

In relation to particular practical problems we have completed the analysis of the tracer data on fatty acid metabolism, in collaboration with Dr. Fredrickson. This study is now being written up for final publication.

Direction of current Research:

The mathematical investigation of biological transport problems is being continued along two lines: (1) The general theory is being studied as a problem in linear analysis. (2) Various particular problems are being studied. Here the immediate emphasis is analysis of counter current exchange system with particular reference to kidney function.

Part B included - Yes

Serial No. NHI-298

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Stephenson, John L.: "Theory of Transport in Linear Biological Systems: I. Fundamental Integral Equation." Bull. Math. Biophys. 22:1-17, 1960.

Stephenson, John L.: "Theory of Transport in Linear Biological Systems: II. Multiflux Problems." Bull. Math. Biophys. 22:113-138,1960.

Stephenson, John L.: "Integral Equation Description of Transport Phenomena in Biological Systems." Proceedings of the Fouth Berkeley Symposium on Probability and Statistics. In Press.

Serial No. NHI-299
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Physics of Ultra-Rapid Freezing of Water, Colloidal Solutions and Protoplasm

Principal Investigator: John L. Stephenson

Other Investigators: George Bray (LKEM)

Cooperating Unit: Laboratory of Kidney and Electrolyte Metabolism (NHI)

Man Years (calendar year 1960)
Total: 1.5
Professional: .5
Other: 1.0

Project Description:

Progress During Past Year: (Began 1954)

The general purpose of this project remains: (1) To investigate the basic physics of the rapid freezing process in water, colloidal systems and protoplasm. (2) To apply this information to the analysis of hydration phenomena in protoplasm. (3) To extend the range of application of freezing and drying as a method of fixation and preservation of biological material.

During the past year a project in electron microscopy not directly related to the freezing problems was carried out in collaboration with Dr. George Bray of the K & E Laboratory. An attempt was made to fix the medullary region of the rat kidney, (which has so far not been studied successfully, by electron microscopy) in osmic acid solution of various tonicities. The primary purpose was to study the epithelium of the loop of Henle, particularly for differences between the ascending and descending limbs, in order to see if any electron microscopic evidence of differences in function suggested by Kuhn's theories of urine concentration could be discovered. A few specimens in which the papillary region was well fixed were obtained, but good fixation could not be obtained regularly. In these few specimens the capillaries, collecting ducts and thin segments of the tubules were tentatively identified in close juxtaposition to one another, the picture lending support to the general ideas of counter current multiplication, but the data were too incomplete to allow any sequential construction of the epithelium of the loop.

Direction of Current Research:

Work is being continued on the relation of freezing rate to ice crystal formation. At present we are devising a procedure for freezing specimens in tiny chambers in thin methacrylate sheets. These sheets also have embedded in them, next to the specimen a small thermocouple. Thus, a complete record of temperature during freezing and subsequent drying can be obtained for subsequent correlation with electron microscopic study of ice crystal size.

Part B included - Yes

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Individual Project Report
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Part B Honors, Awards, and Publications

Stephenson, John L.: "Fundamental Physical Problems in the Freezing and Drying of Biological Materials", Recent Researches in Freezing and Drying, pp. 121-145. Edited by A. S. Parkes & Audrey U. Smith, Blackwell Scientific Publications, Oxford, 1960.

Stephenson, John L.: "Ice Crystal Formation in Biological Materials During Rapid Freezing", Annals of the New York Academy of Sciences, 85:535-540, 1960.

Comment on "Theory and Practice of Freeze Drying", Terence W. G. Rowe Ibid 679-681.

Serial No. NHI-300
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Biochemical Effects of Ultrasonic Waves

Principal Investigator: Alfred Weissler

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: None
Total: 1.0
Professional: 1.0
Other: None

Project Description:

Progress During Past Year:

The investigation of the effects of ultrasonic waves on a dilute aqueous solution of hemoglobin was completed and published. One of the interesting results was that the destruction of hemoglobin is caused by the small amounts of nitrous and nitric acids produced sonochemically from the dissolved air and the water. Hydrogen peroxide and hydroxyl free radicals do not seem to be as important in this case as in others, possibly because hemoglobin can destroy peroxide.

A second publication this year contained the results of a study on sonochemical reaction yields. Comparison of the "free radical" and "molecular" yields with those of radiation chemistry showed that ultrasound resembles alphaparticles in chemical effects, rather than gamma or x-rays.

The interest in sulfhydryl compounds has continued. Consultative advice given to other projects in the National Heart Institute has recommended the use of sulfhydryl protective reagents (such as cysteine or mercapto-ethanol) for minimizing loss of ATPase activity during sonic fragmentation of myosin, and for decreasing enzyme losses in erythrocyte membranes during sonic disruption of the red cells. The success of this simple procedure indicates that an understanding of the chemical effects, which occur with intense sound waves in aqueous systems, will have widespread practical importance.

Cysteine was selected as a model substance for studying the action of ultrasound on sulfhydryl compounds. Preliminary experiments, using a colorimetric analytical method for the SH group, showed that oxidative destruction occurred initially. On continued treatment, however, there seemed to be regeneration of sulfhydryl. Further work is awaiting a decision on the suitability of the analytical method.

Another amino-acid, tryptophan, has been studied sonochemically in some detail. In this case, fluorescence analysis proved to be very useful. A 10^{-5} molar solution of tryptophan was exposed to 400 kilocycle ultrasound plane waves, about 8 acoustic watts being absorbed in 25 ml of solution in a 32x200 mm. pyrex test-tube. The fluorescence spectrum was recorded after successive treatment periods, using an activation wavelength of 286mu. The emission peak height at 365 mu was taken as a measure of the tryptophan concentration.

Runs were made with tryptophan solutions at pH 4, 7, and 11, and with the solutions saturated with argon, oxygen, or air. In these dilute solutions, the destruction of tryptophan follows first - order kinetics, after a short initial period. Also, the rate of destruction is fastest in oxygen, next in argon, and slowest in air. This contrasts with the results found previously for hemoglobin, and indicates that sonochemically-produced nitric acid cannot be the principal agent responsible for the attack on tryptophan. Instead, OH and possibly HO_2 radicals are involved.

It was also found that a new peak appeared in the fluorescence spectrum of tryptophan after ultrasonic treatment. This showed up around 400 mu, with an optimum activation of 325 mu. It is due to a substance produced when tryptophan is destroyed, possibly by opening of the heterocyclic ring to give a molecule of the formylkynurenine type. (Such a reaction is known to be caused by intense ultraviolet illumination of tryptophan solutions for long periods.) Work is continuing on the identification of the substance responsible for the new peak.

Exploratory experiments on the use of spectrophotofluorimetry for quantitative measurement of the effect of ultrasonic irradiation on tyrosine and riboflavin gave encouraging results also.

During the year, discussions took place with other members of this Laboratory on the subject of sound velocity and absorption measurements at ultrasonic frequencies. At the writer's request, a pulse unit for ultrasonic absorption work was constructed from a published design, but it was not found satisfactory. Consideration has also been given to adapting the gas chromatograph sound-velocity detector for use in measuring sound velocity in liquids. For many years, ultrasound velocity and absorption in fluids have been used both for characterization and for studying molecular structure, in a manner comparable to the use of light velocity (i.e., refractive index) and absorption (spectrophotometry). In this Laboratory, the objective would be to extend the applications in biochemical materials.

Direction of Current Research

Identification of the products of ultrasonic destruction of tryptophan, at different pHs, is the most immediate problem. Other work will be the long-planned study of conditions which may modify the loss of enzyme activity which often occurs during ultrasonic disruption of cells; the starting point will be with sulfhydryl enzymes and SH protective agents. Ultrasonic velocity and absorption measurements in biological materials are also contemplated, if circumstances permit. A fourth area is concerned with the mechanism of cell damage in protozoa by ultrasound, with special reference to the disulfide-sulfhydryl equilibrium in the cell membrane.

Part B included - yes

PHS-NIH
Individual Project Report
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Part B: Honors, Awards, and Publications

Weissler, A.: "Some Sonochemical Reaction Yields", J. Acoust. Soc. Am., 32, 283-4 (1960).

Weissler, A.: "Effects of Ultrasonic Irradiation on Hemoglobin", J. Acoust. Soc. Am., 32, 1208-12 (1960).

Serial No. NHI-301
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Development and Evaluation of an Aqueous Glass for Studies of Phosphorescence of Polar Compounds.

Principal Investigator: Joseph E. Hayes, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960) Patient Days: None
Total: .5
Professional: .5

Project Description:

Progress During Past Year:

Phosphorescence excitation and emission spectra and emission lifetimes have been determined for a series of substituted benzoic acids in acid and alkaline aqueous solutions with propylene glycol at -89.5°C. (see 1959 Annual Report Phosphorescence in Aqueous Media, NHI-286) and in EPA, a 5:5:2 mixture of ether, isopentane, and ethanol, at -196°C. The series consists of the three position isomers of amino-, hydroxy-, methoxy-, methyl-, fluoro-, chloro-, bromo-, iodo-, carboxy-, and nitro-benzoic acids, as well as benzoic acid itself. The nitro-compounds are of such low activity that they will not be considered with the rest of the series.

The solubilities of p-iodobenzoic acid at pH 1 and of p-carboxybenzoic acid at pH 1 and in EPA are too low to permit their inclusion at the same concentration as the other compounds; consequently they were not examined in these solvents. Otherwise all compounds were tested at a final concentration of 0.0075M in EPA, 1:1 1N $HClO_4$-propylene glycol, and 1:1 0.008N NaOH-propylene glycol. In the latter system, additional alkali was added to the phthalic acids. One can expect a lesser degree of association and solvation in EPA than in the other two solvents, thus the actual molecular species involved is probably different in the three solvents. This will affect the comparisons to follow and probably accounts for the low degree of systematicity in the aqueous systems.

Let us discuss first the behavior of the compounds in the two aqueous systems. There is no detectable phosphorescence from o-fluorobenzoic acid, o-fluorobenzoate ion, nor m-iodobenzoate ion. In the case of m-hydroxybenzoic acid, the acid form exhibits no phosphorescence but a very intense fluorescence as indicated by (a) no observable lifetime and (b) peak emission at a slightly shorter wavelength than the room-temperature fluorescence emission peak. The singly ionized form emits a weak phosphorescence. The m-aminobenzoate ion emits rather weakly. Otherwise, with the exceptions of the phthalic acids which are a special case and the chlorobenzoic acids, the intensity of emission tends to increase with decreasing electronegativity of the substituent, as does the lifetime of emission in the case of the ionized forms. With the undissociated acids, there is somewhat more randomness in lifetime, and a tendency toward a shorter lifetime than that of the corresponding ion. Variations in intensity among the triplets of isomers are fairly random, but with an overall trend toward greatest intensity for the para isomer, particularly with substituents less electro negative than hydrogen where intensity tends to decrease as para, ortho, meta. In summary one might say that there is a tendency for phosphorescent intensity to decrease with increasing acid strength.

In EPA this effect is both more marked and more clear cut. With hydroxy- and iodo- as exceptions, the logarithm of the ratio of intensity para/meta varies as the pK of the para acid. This variation with acid strength or, alternatively, with the Hammett substituent constant, indicates that phosphorescent efficiency is promoted by increasing localization and density of the pi electrons. The shift in absorption maximum from compound to compound is sufficiently slight and regular that the non-linearity of the instrument would produce only a minor error. All the compounds except m-aminobenzoic acid are more active in EPA, often by several orders of magnitude than in the aqueous systems. As would be expected, lifetimes are considerably longer at the lower temperature.

A question of potential importance to comparison of emission intensities of various substances is that of the relationship among lifetime, shutter speed, and effective concentration of excited state. Consider a shutter containing two diametrically opposed 60° ports, rotating at a constant angular velocity. Then if the shutter is just opening to admit exciting light to the sample at zero time, this excitation will continue until a time corresponding to 60° rotation and then be stopped. After a further 30° rotation, during which decay is occurring, the sample is examined for 60° by the emission monochromator; the meter presenting a kind of average value for the intensity. Without a priori knowledge that the excitation route is the inverse of the decay route, no statement can be made regarding identity of the time constants for these two processes. However, at least in the case of the relatively long lived emitters, these two time constants are the same within measurement error.

An electrical analogue was constructed for the excitation-emission process in order to provide a method for independently varying shutter speed, and the two time constants involved.

Use of the analogue indicates that when the two time constants are the same and when the lifetime is long compared to the time for a shutter-port opening, wide variations in shutter speed have only a slight effect on meter reading. This effect is an increase in sensitivity with increasing shutter speed. With shorter lifetimes, the effect becomes somewhat more pronounced. There is no simple way of converting data to a standard basis in this respect.

Direction of Current Research:

The data on substituted benzoic acids are being analyzed preparatory to publication. A survey will next be made of a similar series of substituted phenols and of a range of compounds of greater biological interest.

Part B included - No

Serial No. NHI-302
Laboratory of Technival Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Analysis of Absorption Spectra in Terms of Individual Bands by means of a Special-Purpose Analogue Computer.

Principal Investigator: Joseph E. Hayes, Jr.

Other Investigators: None

Cooperating Units: Spectra are determined in a Cary Model 11 Spectrophotometer in the Laboratory of Chemistry of Natural Products, National Heart Institute.

Man Years (calendar year 1960) Patient Days: None
Total: .2
Professional: .1
Other: .1

Project Description:

Progress During Past Year: (Began 1958)

During the past year absorption spectra of a series of aromatic nitro compounds previously synthesized or collected and purified have been determined in aqueous solution and subjected to analysis in terms of individual absorption bands. The analyses were performed using a special-purpose analogue computer developed in this laboratory (Repetitive Analogue Computer for Analysis of Sums of Distribution Functions: F. W. Noble, J. E. Hayes, and M. Eden, Proc. IRE 47, 1952 (1959)) The series of compounds so far collected consists of the three position isomers of nitroaniline, nitrophenol, and nitrobenzoic acid and the six possible dinitrophenols. The three nitrobenzaldehydes and the three nitroanisoles will be run only in non-aqueous media. Spectra of the same compounds in a non-polar solvent (trimethylpentane) must be determined and analyzed before attempts at interpretation can be made. Use of a hydrocarbon solvent can be expected to alter the spectra in three ways. First, it solvates the organic compound less than water, affecting any absorption due to solvated forms. Second, it reduces association thus reducing a restriction on molecular vibration

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and increasing the intrinsic resolution of the spectra. Third, with decreased dielectric constant of solvent, bands due to absorption in the benzene ring and those due to absorption in the unconjugated substituent groups tend to draw away from each other. This not only increases the resolution, but also gives a way of assigning a band to a given chromophore in the molecule.

Direction of Current Research:

A further series of compounds, consisting of the three position isomers of amino-, hydroxy-, methoxy-, methyl-, fluoro-, chloro-, bromo-, iodo-, and carboxy benzoic acids is on hand. These are to be subjected to the same study as the nitro-compounds.

Dr. H. Fales of the Laboratory of Chemistry of Natural Products has collected a series of gas-chromatographically pure alkane hydrocarbons for a similar study of infra-red spectra. This series consists of the normal hydrocarbons C_6-C_{11}, C_{13}, and C_{15} along with 2,2,4-trimethylpentane, 2-methylhexane, 2,2,3-trimethylbutane, and 2,2-dimethylpentane.

Further work will proceed as indicated above. It is expected that correlations of at least a qualitative nature will be found between absorption spectra and molecular structure, in a resonance sense, in the case of ultra-violet-visible spectra. In this case the studies would be extended to compounds of greater biological interest. For the infra-red spectra, it is hoped that the improved resolution, leading to cancelling of shifts in band position due to overlapping, will permit more precise assignment of infra-red bands. In addition, information about intramolecular vibration interactions should be obtainable from study of the variation of, for example, integrated band intensity for the CH_2 stretch with chain length in the straight chain hydrocarbons. Finally, it is hoped to assign a frequency to the CH stretch, a low-intensity band normally so "buried" under high-intensity bands that it is not found by simple inspection.

Part B included - No

Serial No. NH1-303
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Determination of Correction Factors for Linearization of Spectrophotofluorimeters and Spectrophosphorimeters.

Principal Investigator: Joseph E. Hayes, Jr.

Other Investigators: None

Cooperating Units: None

Man years (calendar year 1960) Patient Days: None
Total: .2
Professional: .2

Project Description:

Progress During Past Year: (Began 1960)

Spectra, either excitation or emission, obtained in commercially available spectrophotofluorimeters or spectrophosphorimeters are subject to a systematic error. This is due to these being single-beam rather than double-beam instruments. Thus, since the energy output of the exciting lamp increases sharply with increasing wavelength, an excitation peak will appear at a longer wavelength than is correct. This is because the critical quantity, amount of light absorbed, is proportional not only to the absorbancy of the sample solution but also to the intensity of light available for absorption. Similarly, the emission spectrum tends to be shifted toward the wavelength of maximum sensitivity of the photomultiplier. For the tube types normally used, this is 350-400 mu. Existing methods of determining correction curves to be applied to observed spectra are imprecise and time-consuming.

There is in principle a rapid and convenient method of determining such curves, suggested by Weber and Teale, and by Slavin. Given a fluorescent substance capable of absorbing throughout the range over which calibration is desired and which emits at a yet longer wavelength, one can take advantage of the fact that the absorption spectrum and the excitation spectrum should be identical. In other words, the correction curve desired is that which will convert the excitation spectrum of such a substance to its absorption spectrum. There are problems concerned with sample geometry which are not yet fully solved and which involve both intensity and wavelength. Trials are being made both with dilute solution and with quite concentrated solution in a thin layer. The substance used is a highly purified sample of the fluorescent dye Rhodamine B, which absorbs from 200 to 600 mu and has its emission maximum at an apparent position of 585 mu. Data thus far obtained agree with a calibration done according to White's

method using the Hatchard and Parker ferrioxalate actinometer in the range 230-330 mu. Above 330 mu there is a great deal of scatter which is almost certainly due to the much lower extinction of the dye in this region leading both to less accurately known extinctions and to greater sensitivity to stray light. This method of calibration clearly applies only to the excitation side of the instrument.

Replacement of the sample by a suitable scatterer would permit transfer of the calibration to the emission side, over the wavelength range covered. Thus far, six commercial samples of glycogen (suggested by Weber and Teale) and a sample of DuPont Ludox (silica spheres) have been tried as scattering solution. All of them are unsuitable, since they possess absorption as well as scattering.

Direction of Current Research:

Research is currently directed toward improvement of the precision of calibration of the excitation side, search for other suitable dyes, and search for a suitable scatterer.

Part B included - No

Serial No. NHI-304
Laboratory of Technical Development
National Heart Institute
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Study of the Rate of Binding of Fatty Acids to Human Serum Albumin

Principal Investigator: Joseph E. Hayes, Jr.

Cooperating Units: Laboratory of Cellular Physiology and Metabolism
Hans Ott

Man Years (calendar year 1960) Patient Days: None
Total: 0.1
Professional: 0.1

Project Description:

Progress During Past Year: (Began 1960)

In order to determine whether the rate of adsorption and desorption of fatty acids to serum albumin could be the rate-limiting step in fatty acid transport by that means, experiments were undertaken to measure the rate of binding of myristic, palmitic, and oleic acids to the protein. Since the equilibrium constant for the binding is known, this would also permit calculation of the rate constant for release.

A Chance stopped-flow apparatus was used, taking advantage of the difference in extinction at 290 mu between protein and protein-fatty acid in alkaline solution. Although resolution to perhaps 20 msec. could thus be obtained, the binding reaction was too rapid for accurate measurement. Thus, this is probably not the factor governing the rate of transport.

Direction of Current Research:

The data obtained are being prepared by Dr. Ott for publication.

Part B included - No

Serial No. NHI-305
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Coriolis Flowmeter

Principal Investigator: Peter L. Frommer

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960): Patient Days: None
Total: 0.05
Professional: 0.05
Other: None

Project Description:

Progress During Past Year:

The coriolis force, which is the extra force exerted on a moving structure by the movement of another mass along this structure, can be utilized for the measurement of mass flow. As described in the 1959 annual report, an experimental blood flowmeter was built, and tested with water; it produced full scale reading for 100 gm/sec flow with linear response and five per cent reproducibility.

However, with the improvements in blood flow metering devices utilizing electromagnetic and ultrasound transducers and with the advances in industrial mass flow metering devices, further work on a cariolis force blood flowmeter did not seem indicated and work on this project was discontinued.

Direction of Current Research: Discontinued

Part B included - No

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Serial No. NHI-306
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Cardiac Indicator Dilution Curves with Ascorbic Acid, Using Intravascular Sensing Electrodes.

Principal Investigator: Peter L. Frommer

Other Investigators: Eugene Braumwald, (Heart-Surgery)
William Pfaff, (Heart Surgery)
Dennis Prager, (COSTEP Student)(LTD)
Section on Cardiology of the Surgery Branch

Cooperating Units: Heart-Surgery

Man Years (calendar year 1960) Patient Days: None
Total: 1.0
Professional: 0.8
Other: 0.2

Project Description:

Progress During Past Year:

The conventional dye dilution methods used in cardiac catheterization are hampered by the loss of blood, by the appearance of dye in the skin, and by distortions in the curves caused by mixing in the smapling system. Therefore, a study was undertaken to perfect and apply techniques with electrochemically active indicators which overcome these problems by being sensed in situ and by being free of undesirable side effect.

The oxidation of ascorbate upon its contact with an intravascular platinum anode produces a current between that electrode and a cutaneous silver reference electrode which is linearly related to the ascorbate concentration. Initially, this technique worked erratically, with great variations in sensitivity, with large artifacts coincident with the pulse, and with no means for calibration.

Experiments were undertaken using a 1 mm. diameter platinum anode and a large chloridized silver reference electrode immersed eccentrically in a rotating beaker containing blood. It was found that when voltage was applied to the electrodes, there was a large inrush of current to the electrolytic capacitance of the blood. It was 5 to 30 minutes before reasonable stability was reached and throughout this period there was a proportionate decrease in response to ascorbate; this suggested that other

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factors were also present that seemed to act by diminishing the effective electrode surface area. Most studies were done at +0.9 or +1.0 volts, which are points on the plateau of the ascorbate oxidation wave, but similar results were obtained throughout the positive voltage range. In subsequent studies it was found that reasonable stability could be achieved much more quickly by initially applying an overvoltage, such as 1.34 volts, for a half minute before dropping to the 1.0 volt measuring level.

It was hoped that repeated reversal of the electrode processes by alternating the applied voltage would prevent this long term drift, or that the intermittent application of voltage would minimize this effect. Symmetric and assymetric square waves, sine waves, and low duty cycle positive pulses and ramps were studied and found to increase stability. At the frequencies studied, the transient currents to and from the electrolytic capacitance of the blood were several orders of magnitude greater than the ascorbate oxidation currents. However, the average or net current flow was very sensitive to ascorbate concentration and linearly related thereto, but with a proportionality factor which was not constant.

A proper choice of alternate polarity voltages (e.g. +0.9 and -1.6 volts for 2 milliseconds each) produced around zero net current, and ascorbate oxidation increased the net current. This mode of operation offered the closest approach to stability. Response was linear, with 1 mgm ascorbate per ml. of blood producing about 20 microamperes; changes of 2 microgram per ml could be detected in vitro. However, because oxygen reduction occurred during the negative voltage phase, a prohibitively large variable was introduced against the clinical use of this method. Brief positive square or ramp voltage pulses, relatively widely separated from each other (e.g. 1 volt 2 millisecond pulses 25 times a second) offered intermediate stability between the d.c. and the alternate polarity methods and this system was insensitive to oxygen tension. With one electrode in intracardiac position and the other on the skin, the variable voltage methods induced fatal arrhythmias in barbiturate anesthetized dogs and in their present form, the variable voltage methods are unsatisfactory for intracardiac clinical use. A constant applied voltage applied to similarly located electrodes has never produced extrasystoles or arrhythmias either in animals or in clinical use. With the d.c. method, electronic protection has been provided to limit short circuit currents to 600 microamperes, well below the rheobase for cardiac stimulation.

While the cause of the pulsatile artifacts has not been discovered, they can be minimized by minor repositioning of the electrode.

The ascorbate method is now being used routinely for dilution studies not requiring absolute quantification of amplitude. Electrodes have been incorporated on intracardiac catheters; other electrodes have been prepared that can be threaded through an indwelling arterial needle. Ascorbate is administered as its buffered sodium salt in doses between 50 and 300 mgm at a concentration of 100 mgm per ml.; signals range from 0.1 to 50 microamperes. The method permits a versatility of sampling and injection

sites, both intracardiac and peripheral, which have lent themselves to a number of new techniques in addition to the standard ones.

Direction of Current Research:

Primary attention is being given to applying the versatility of the ascorbate method to such clinical problems as the quantitation of mitral regurgitation and the more precise localization and quantitation of shunts.

An attempt will be made to minimize the artifacts coincident with the pulse by stabilizing the electrode geometry by positioning both the platinum and reference electrodes on the catheter in close proximity to each other.

Greater stability and reproducibility of results will be sought by study of chemical and electrical cleaning and pretreatment of the electrodes.

Quantifying results will be attempted again. With the present relatively unstable method, a small blood specimen will be drawn shortly after each complete curve and a comparison between the current at that time and the result of chemical assay will establish the calibration for that time. Because absolute quantitation is generally applied to curve areas, methods will be studied which might achieve stability but which sacrifice rapid response and faithful representation of the curve. Very low frequency (e.g., 1 per second) ramp or square waves offer promise; diffusion limiting membranes will also be considered.

If a technique can be developed for the stable amperometry of ascorbate in so complex and unfavorable a medium as blood, other applications of the technique will be examined.

Part B included - yes

Serial No. NHI-306
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Frommer, Peter L.: Cardiac Indicator Dilution Curves with Intravascular Platinum Electrodes - an AC Polarographic Method, Digest of Papers, 13th Annual Conference on Electrical Techniques in Medicine and Biology, page 18, Lewis Winner, Publisher, 1960.

Serial No. NHI-307
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NHI
Individual Project Report
Calendar Year 1960

Part A

Project Title: Quantitation of Mitral Valve Regurgitation

Principal Investigator: Peter L. Frommer

Other Investigators: Eugene Braumwald, (Heart-Surgery)

Cooperating Units: Section on Cardiology of the Surgery Branch (Heart)

Man Years: (calendar year 1960) Patient Days: None
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:

Progress During Past Year:

As reported last year, the quantitation of mitral regurgitation was to be attempted by quantifying the increased optical density over the left atrium in serial angiograms following left ventricular injection of contrast medium. In vitro tests in which contrast material was added to a pool of water with pre-existing areas of variable density yielded unfavorable results (best reproducibility 10 per cent; least error 25 per cent); in vivo results would be considerably worse.

The time and effort necessary for such measurements were considerable and the prospects for success were dim. The use of ascorbate as an indicator substance which can be sensed in situ offered new hope for quantifying regurgitation by the method proposed by Lacy, et al, and attention was focused on developing the ascorbate method. (see separate project report)

Minor modifications were done on the electrical analog for indicator dilution in the heart and studies were done to show qualitatively the effects of incomplete mixing of blood within the heart chambers.

Direction of Current Research:

Using the ascorbate indicator method, quantitation of regurgitation will be attempted by the method suggested by Lacy, et al, with minor modifications.

With left ventricular injection and sampling in the mitral orifice, points are obtained during systole and diastole for the regurgitant and for the regurgitant plus forward outputs, respectively. A comparison of the areas under these interpolated curves establishes a simple relation between regurgitant flow and cardiac output. The only assumption is that a true average is obtained for indicator concentration in the blood flowing through the mitral valve orifice during each systole and diastole; complete mixing within the chambers is not necessary.

Attention will be directed toward the development of a trans-septal catheter which permits left ventricular injection while simultaneously placing a platinum sensing electrode into the mitral orifice. A major problem will be placing and keeping the electrode in the valve orifice or at a point at which there is a similar concentration of indicator.

Part B included - yes

PHS - NHI
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Frommer, Peter L.: A Passive Element Analog of Indicator Dilution by the Heart, 13th Annual Conference on Electrical Techniques in Medicine and Biology, Lewis Winner, Publisher, New York, 1960, page 78.

Serial No. NHI-308
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Development of Methodology for the Effective Application of Gas Chromatography to Biochemical and Medical Research, and Applications to this Methodology.

Principal Investigator: Arthur Karmen

Other Investigators: R. L. Bowman, P. L. Frommer, H. Tritch (LTD), R. J. Gordon, J. Bragdon, J. Avigan, D. Steinberg, M. Vaughan, E. Feigelson, M. Horning, P. Vagelos, D. Martin, D.Goodman(LCPM), J. Pisano (LCB), H. Saroff (NIAMD), H. Kayden & A. Dumont (New York University College of Medicine)

Cooperating Units: Laboratory of Cellular Physiology and Metabolism, Laboratory of Clinical Biochemistry, Institute of Arthritis and Metabolic Diseases, New York University College of Medicine.

Man Years (Calendar Year 1960): Patient Days:
Total:
Professional:
Other:

Project Description

The work of this year has been a continuation of the research long the lines of (1) development of methodology for biochemical and medical research problems; (2) applications of this methodology; (3) lipid metabolic studies.

Progress During Past Year:

I. Methodology (A. Karmen, L. Giuffrida, I. McCaffrey)
   A. Radioassay of C^{14} labeled compounds analyzed by gas chromatography.

A major portion of the development program has been involved with perfecting the method of radioassay of compounds analyzed by gas chromatography reported in last year's summary.

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1. The effectiveness of the method of collection of eluted materials on short sections of gas chromatography columns has been confirmed.

2. Collection on a short section of gas chromatography column composed of anthracene crystals for direct, cumulative, continuously monitored scintillation counting has been accompanied by some difficulties. Prevention of condensation of fatty acid esters in tubing leading from the column has required keeping all tubing hot. The hot gas impinging on the surface of the anthracene impairs its efficiency. The cumulative counting record demonstrates this effect by excessive "tailing" of the components as they enter and move through the heated layer of anthracene into the more efficient lower layers. A working solution to this problem has been through the use of cooling of the anthracene filled vial, which has given more uniform results, and higher efficiency. It is possible to analyze mixtures containing only several counts/time constant with about 50 per cent efficiency, and negligible backgrounds. Extending this method to the radioassay of labeled steroids analyzed by gas chromatography has been accompanied by still other problems. Steroids are more easily condensed, which required still hotter connecting tubing. They tended to condense on the first layers of anthracene they contacted, and external cooling of the vial was insufficient to restore these to efficiency. The result was markedly lowered apparent recovery of radioactivity. To solve this problem, a device for collection whose action may be best likened to that of a vacuum cleaner has been evolved. The heated column effluent and substantially larger quantities of cool air are sucked through glass funnels containing anthracene crystals, while the steriods are retained in the anthracene. Close to total recoveries and predicted efficiencies have recently been obtained using this device.

Direction of Current Research:

It is anticipated that further and continuous use of this method will indicate modifications necessary for greater reproducibility as well as more convenient integrated systems of columns, mass detectors and radioactivity detectors.

It is also intended to investigate the possibilities of extending the technique to trituim labeled compounds.

Part B included - yes

B. A sensitive, non-radioactive, non-destructive Detector for Gas Chromatography (A. Karmen, R.L. Bowman, P. Frommer)

Operation of an integrated system for determining the radioactivity and, at the same time, determining the mass or quantity of the eluted material lead to the early choice of the use of the argon ionization detector described by Lovelock as mass detector. This detector, among the highly sensitive detectors, was somewhat unique in that it did not destroy the components detected. It was noted, however, that the radioactive sources leaked sufficient radioactivity into the collection device to elevate the backgrround radioactivity to thousands of counts per minute, as well as contributing to the background through penetrating radiation.

Development of a non-destructive, non radicactive, highly sensitive detector was therefore undertaken, and a detector was developed which has these properties. The detector is based on the use of a self-sustained, low current, electric discharge excited in argon or helium by the application of a high intensity electrostatic field. The detector is extremely sensitive to changes in vapor concentration, with detection sensitivity at least as good as any that has been reported. Sensitivities of 1 molecule /10^9 carrier have been easily obtained.

Over a narrow range of current, this discharge is quite stable. Utilization of the method reported last year of electronically varying the field intensity necessary to maintain the current through the discharge constant was found to be useful, if not the most useful, method of operation of this detector.

The use of this highly sensitive detector has made possible the analysis and radioassay of small quantities of radioactive material with high specific activity. Biological experiments in which this ability is useful have not as yet been forthcoming, since the usual experiment, even those in which no carrier non-radioactive material has been added, either has required that relatively large quantities of unlabeled material be analyzed for their small yield of radioactive substance, or that small quantities of material bearing small quantities of radioactive material be analyzed in the presence of large quantities of other contaminating materials. The detector is not well suited for analyses in which large quantities of material are involved, because of instability at high vapor concentrations and thus is less than ideal for this application.

Although the problem as outlined may be present be solved by the use of more than one detector among any of several now available adaptation of any single one to most problems likely to be encountered requires further investigation.

C. Detector for Liquid-Liquid Chromatography (A. Karmen, H. Tritch) 1 month

Lack of a general sensitive detector has presented the full utilization of the liquid-liquid chromatographic technique, which, conceivably, might be extended, for example, to high resolution, narrow diameter columns, if a sensitive detector were available.

For use in lipid analyses, the detector should be able to detect and quantify lipid materials diluted by large quantities of solvent, whose chemical properties are quite similar to the lipids to be detected and which may be varied (e.g. "gradient elution") during the course of an analysis. This latter criterion makes operation of a detector in which a property of the solvent is monitored difficult.

On the basis that many lipids differ appreciably involatility from the chromatography solvents, a prototype detector was constructed in which the column eluate was deposited on a moving belt conveyor system, and, successively: 1) the solvent evaporated; 2) the material subjected to the heat and ionizing properties of a hydrogen flame ionization detector, such as is used in gas chromatography.

The continuous conveyor system concept was demonstrated to be feasible, and sensitivities were found to be quite high. It was equally evident that a discontinuous system, whereby discrete aliquots were delivered to the flame at regular intervals shower promise of greater versatility. Higher sensitivity, at the price of a diminished speed of response could be obtained.

"Minor" technical difficulties have slowed development of this device into a practical instrument. The primary requirements are for 1) a method of pipetting or painting the column eluate on the conveyor surface for delivery to the flame, which produces a uniform coating from which solvent may be evaporated. 2) A method for delivery of the material to the flame such that it is not exposed to intense heat until it reaches the flame, and, on the other hand, does not enter the flame area through a port which gives atmospheric debris and organic vapors access to the flame.

D. Project (A. Karmen, L. Giuffrida, I. McCaffrey)

A method for improvement of the sensitivity of ionization chambers as sensing devices for carbon ^{14} in a gas chromatography column effluent.

A method for increasing the current yield per radioactive disintegration would permit extension of the use of ionization chambers to the gas chromatography effluent C^{14} detection problem. Present chambers require such large quantities of radioactivity as to prevent their use in biological experiments. A method of increasing the current yield was

suggested by the efficacy of the argon ionization detector. In this detection method, current flow is measured in argon, a constant amount of radioactivity, high voltage, and a variable organic vapor concentration. It would seem feasible to substitute radioactivity for organic vapor as the variable. By adding a fixed concentration of organic vapor, large in comparison to the material leaving the column, the current should reflect only the radioactivity present in the column effluent. The current should be several orders of magnitude greater than that obtained from ordinary ion chamber gas mixtures.

Preliminary experiments have revealed that standard ionization chambers react to the entry of unlabeled organic vapors in fashion somewhat specific for the compounds entering. This effect, which may be related to surface potential changes at ehe electrodes, is presently being investigated, to explore the possibilities of its usefulness as a detector as well as the possibilities of eliminating it.

E. Separation of lipids by paper chromatography (A. Karmen, L. Giuffrida, I. McCaffrey)

To provide a convenient micro method for separation of lipids into the various classes, the investigation of the paper chromatographic technique on silicic acid coated paper was continued.

1. Chemical detection methods were adopted and developed for the identification of the compounds on the paper.

2. Reproducible results in terms of reproducible retardation factors on silicic acid papers did not appear to be forthcoming. This was attributed to variable amounts of water on the paper. The relative retardation factors, on the other hand, were quite reproducible. In order to make a system such as this practical, efforts were directed toward finding solvents which increased the separation factors between the various classes of lipids. These efforts have met with some success. e.g.

The separation of cholesterol esters from triglycerides was difficult, although the separation of tripalmittin and other fully saturated triglyerides from cholesterol esters was easily accomplished. Separation of the unsaturated triglyerides from cholesterol esters was accomplished by including small concentrations of a dehydrating agent, either absolute alcohol or acetic anhydride in the hexane used for separation. This was applied. (E. Feigelson, A. Karmen, D. Steinberg: Studies of Fatty Liver following Norepinephrine.) It is now possible to obtain wide separation.

1. Wide separation of triglycerides from diglycerides and monoglycerides using another solvent system. This was applied to studies of triglyceride synthesis in adipose tissue. (D. Steinberg, M. Vaughan, M. Margolis: Studies of Triglyeride Synthesis in Adipose Tissue.)

2. Separation of all glycerides from phospholipids.

II. Applications

A. Analysis of Amino Acids by Gas Chromatography (A. Karmen, L. Giuffrida, I. McCaffrey, H. Saroff (NIAMD)

The problems of preparation of volatile derivatives of amino acids were studied. Attempts to analyze amino acid esters met with highly inconstant results, and it was decided to acylate the amino nitrogen as well as preparing the ester. N-trifluoro acetyl amino acid esters were prepared.

Many amino acids were chromatographed in this form, and only tryptophane, among those studied, seemed entirely to resist chromatography by this method.

Several amino acids, such as arginine and tyrosine, however, yielded multiple derivatives. The conditions necessary to eliminate this difficulty were studied but with as yet incompletely satisfactory results.

Exploration of what type of analytical column was most satisfactory was undertaken and polyester columns of the type used for fatty acid analysis was found to be eminently suitable. Whereas the various polyesters showed minimal differences in behavior in fatty acid analyses, the differences in amino acid analyses were striking. More "polar" polyesters retained many amino acid esters for excedingly long time periods. The use of non-polar phases has as yet been incompletely explored.

Detector behavior toward these derivatives was also studied. The presence of the fluorine tended to make quenching agents of the derivatives in ionization detectors. As a result, the sensitivity of the RF detector was highest, the sensitivity of the hydrogen flame detector somewhat less; the behavior of the DC discharge detector in argon, and the argon ionization detector was sufficiently erratic and voltage dependent to suggest their use as qualitatively discriminatory detection devices. Manuscripts submitted (5)

Because of the difficulties inherent in preparing single N-substituted derivatives from individual amino acids, methods of increasing the volatility of amino acid esters (no N-substitution) were reinvestigated. A trial scheme whereby ammonia was added to the carrier gas proved successful. Each of the amino acids that was analyzed easily (without complications) as N trifluro acetyl esters, was analyzed injected as the

ester hydrochloride by this scheme. The hydrogen flame detector was found to be tolerant to the addition of ammonia, without appreicable loss of sensitivity.(submitted for publication)

Projected studies:

1. Study of column conditions necessary to analyze the recalcitrant amino acids is planned.

2. Extension of this technique and modifications thereof to the study for which it was conceived - the analysis of amines of biological importance is also planned for the immediate future.

B. Analysis of porphyrins by gas chromatography (J. Winkelman, A. Karmen)

We are studying a gas chromatographic separation procedure for porphyrins. In pilot experiments we have been able to volatilize porphyrins at greater than 400 degrees C. Preliminary results have shown that several tetraphenylporphines and derivatives move on gas chromatography columns and withstand the high temperature without decomposition.

The vapor of this class of compounds was found to fluoresce. We also intend to characterize the fluorescent spectra of several porphyrins in the gaseous state, which will be a significant aid in the purity analysis of porphyrin samples and enlarge the understanding of factors involved in the rarely found fluorescence of large heterocyclic organic molecules.

III. Metabolic Studies.

A. Studies of Cholesterol Biosynthesis (A. Karmen, L. Giuffrida, I. McCaffrey, D. Steinberg, J. Avigan, D. Goodman)

The success of Dr. E. Horning and coworkers with analysis of steriods, suggested the application of gas chromatography in a similar way to the separation of the compounds on the biosynthetic pathway to cholesterol. Performance of this study involved a thorough study of columns and conditions for separation of these materials as well as for their radioassay as described in the methodology section of this report. This study is very much now in progress.

B. Studies of Fat Absorption (A. Karmen, L. Giuffrida, I. McCaffrey, J. Bragdon, G. Schultz, H. Kayden, A. Dumont)

Preliminary studies of chyle chylomicrons and serum chylomicrons of rats and serum chylomicrons of man indicated that following fat feeding, the fatty acid composition of the chyle and serum chylomicrons closely resembled that of the fed fat, despite reports to the contrary. These results have been published (7).

The possibility of extending this study to human chyle lipoproteins was made possible by an unrelated study being carried out at New York University. Pancreatic enzymes in chyle in relationship to pancreatitis were being studied, and in the course of this study, chyle ducts were being cannulated. A study of chyle lepoproteins following feeding of fat was therefore organized, whereby the chyle samples were collected in New York and fractionated by ultracentrifuge initially by Dr. Bragdon, and subsequently by Dr. Kayden in New York. The fatty acid composition of the fractions were determined in our laboratory. The results may be summarized as follows:

Following fat ingestion, the fatty acid patterns of the chyle chylomicrons cones to resemble that of the ingested fat almost exactly. The fatty acid patterns of each of the other lipoprotein fractions also changed in the direction of that of the fat fed. The change was most evident in the low density beta lipoproteins and in the albumin fractions. Attempts were made to separate each of the chyle and serum lipoprotein classes into their component lipid classes to determine whether the extent of the change in the fatty acid pattern of the whole lipoprotein merely reflected 1) the extent of absorption of the fed triglycerides into that lipoprotein and 2) the fraction of the total lipids the recently absorbed triglycerides represented.

A clear cut answer to this question was not obtained because of a succession of experimental difficulties including instability of some of the unsaturated acids through the multiple separations. It was evident that different degrees of variability of the fatty acid patterns of each of the lipids of each of the lipoproteins was obtained. In most cases this was in the direction of the fed fat.

The time course of the changes in lipoprotein composition was such that the effect of feeding was maximal some 8-12 hours after the meal. The effect diminished following this, but appeared to be still evident as long as 48 hours after the meal. This was taken as indicative of the futility of mass studies (e.g., in "health and disease"), of fatty acid compositions of any serum or chyle fractions, ultracentrifugal or chemical, unless rigid, long term control of diet precedes the study, or unless the point of the study were to determine recent dietary habits.

This work was presented at the meeting of the Society for Clinical Investigation (5/60). Final manuscript is in preparation.

The observations that the composition of the low density, beta lipoproteins, and the albumin fractions, at least, resembled fed fat was shown not to be due to in vitro artefact. Incubation of post-feeding chylomicrons with fasting serum, and incubation of "fasting chylomicrons" with post-feeding serum-minus-chylomicrons revealed no significant interchange of fatty acids, and justified the use of the ultracentrifugal technique. Manuscript in preparation with Dr. Bragdon.

C. Fatty Liver in dogs following administration of norepinephrine (E. Feigelson, D. Steinberg, A. Karmen)

Mechanism of formation of fatty liver in dogs following administration of norepinephrine to dogs has been found to cause acute deposition of fat in the liver. The mechanism of this effect was somewhat delineated by study based on gas chromatography of the fatty acid composition of the triglycerides of the adipose tissue, the free fatty acids of blood, and of the fractionated lipids of the liver. By sequential gas chromatographic analyses of these fractions it was possible to obtain suggestive evidence of transport of the fatty acids from the adipose tissue via the FFA to the liver as the mechanism of the fatty liver produced, rather than increased synthesis or redistribution of the fatty acids in the liver. (submitted for publication)

D. Metabolism of hydroxy fatty acids (R. Gordon (LCPM), A. Karmen)

Study of metabolism of ricinoleic acid was undertaken to obtain more data 1) concerning the metabolism of hydroxy acids; 2) concerning the pharmacologic action of ricinoleic acid.

Although the esters of hydroxy acids - hydroxy stearic and hydroxy oleic ricinoleic are relatively non volatile, application of the techniques used for steriod analysis by gas chromatography yielded a satisfactory analytical technique.

Intragastric administration of castor oil to rats was found to result in absorption of ricinoleic acid into the chyle. Chronic feeding of castor oil to young rats resulted in absorption to the extent that an appreciable portion of rat adipose tissue consisted of ricinoleic acid. (Abstract submitted for publication.)

E. Studies of synthesis of branched chain and odd numbered fatty acids. (M. Horning, D.B. Martin, A. Karmen, P. Vagelos)

Through use of the procedure for continuous, cumulative scintillation counting of the C^{14} in fatty acid esters gas chromatographic effluent, it was possible to show that an enzyme system from mammalian epididymal adipose tissue synthesized branched chain and odd numbered fatty acids when incubated with branched chain and odd numbered precursors. Biochem. and Biophys. Research Commun. 3:101 1960.

F. Identification of urinary catechol amines by gas chromatography (A. Karmen, J. Pisano, S. Udenfriend)

By means of periodate clevage it was possible to prepare volatile aldehyde derivatives of several biological amines, e.g. vanillin from nor-metanephrine. The gas chromatography of this family of aldehydes on various columns were studied. Marked differences among several polyester columns, similar to those described in the amino acid study, were noted.

Analytical conditions were worked out and the possibility of the use of this system for assay was demonstrated.

Through this analytical scheme, it was possible to identify para hydroxy alpha (methyl amino methyl) benzyl alcohol in human urine.

Part B included - Yes

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Calendar Year 1960

Part B - Honors, Awards, and Publications

Karmen, A., Tritch, H.R.: "Radioassay by Gas Chromatography of Compounds Labelled with Carbon-14", Nature, Vol. 186, No. 4719, pp. 150-151, April 9, 1960.

Bragdon, J.H., Karmen, A.: "The fatty acid composition of chylomicrons of chyle and serum following the ingestion of different oils", by Lipid Research, Inc., Journal of Lipid Research, Vol. 1, No. 2, (January, 1960), pp. 167-70

Horning, M., Martin, D.B., Karmen, A., Vagelos, P.: "Studies of synthesis of branched chain and odd numbered fatty acids", Biochem. and Biophys. Research Commun. 3:101, 1960.

Serial No. NHI-309
Laboratory of Technical Development
National Heart Institute

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Development of a Method to Store and Integrate Single Flash Optical Spectra to Provide a Convenient Means of Recording the Emission Spectrum of a Phosphorescent Substance

Principal Investigator: Robert L. Bowman

Other Investigators: None

Cooperating Unit: None

Man Years (Calendar Year 1960) Patient Days: None
Professional: 0.1
Other: .2

Project Description:

Progress During Past Year:

Phosphorescence can be studied by either a continuous system where the subject material is alternately illuminated and examined through a shutter system or by observing the emission following a single illumination period. Shutter systems require very high speed mechanical shutters or elaborate electronic ultrasonic shutter systems. It is considered simpler to provide a single very bright flash and a single shutter opening system. A single delay is required and trial and error phasing can be used to set the shutter opening time. The duration of the phosphorescence will be different for different materials and in many cases will be too short for scanning the wavelength in the time available. As it is not considered necessary to get the true phosphorescent spectrum, but rather a qualitative curve for identification or detection of phosphorescent blank, no great resolution or equal energy response is required. If no interfering peaks are present in the blank the entire phosphorescent spectrum could be integrated for quantification of the unknown.

Photographic recording and densitometric measurement of the plate can perform the required task but the necessity for a survey of methods for producing phosphorescence in biological materials makes this too slow to be practical.

Fluorometric recoveries ran ± 15% of expected. The physical properties of the concentrated solution did not make an equivalent system so that Rhodamine was substituted as it seemed to handle easier and no alkali is needed to keep it in solution. The results of these tests suggested that either the pipetting system was the source of error or the use of concentrated solutions were not suitable for the test as no means of control is available. Drying at the exposed capillary pipette tip has always been a problem that was handled by measuring the liquid column well up inside the capillary and never allowing the sample time to dry before measuring it. Elimination of drying errors was at first attempted by repeating the Rhodamine pipettings in a humid chamber, but even in a small chamber of wet blotting paper some loss was observed when the sample was at the tip. Recoveries were not notably improved.

Revision of the sample pipette to eliminate these errors was next attempted by constructing automatic filling pipettes consisting of a short length (0.5 cm) of 35 micron ID quartz capillary waxed into a larger (200 micron) tube, so that only the inner tube would fill by capillarity and eliminate the need to measure the length of the liquid column. Drying is assumed to take place at the ends without deposition of crystals and transfer is accomplished more rapidly. As the fluorescence recovery tests are difficult and open to additional errors, the recoveries are being tested on sodium recoveries as both methods currently give equivalent errors.

Potassium determinations are essential to the utility of the method and sodium-potassium recovery ratios will assist in testing the method. Sensitivity to sodium is well beyond the requirements, but the potassium sensitivity is borderline, due to difficulty in measuring potassium in the presence of as much as a hundred times as much sodium. A monochromator provides a good Na K separation, but the geometry required for effective use of the monochromator puts stringent requirements on the position and shape of the glow discharge, so that several filter combinations were tried to provide the necessary separation in an interference filter, several thickness of didymium glass, and a glass IR filter failed to eliminate sodium interference. A monochromator consisting of two 60° glass prisms in series and a F3 collimating system was constructed to provide a low resolution high aperature "filter" to eliminate the second order transmissio of the interference filter. At the moment it is not clear whether this system will provide the desirable compromise between resolution and convenience.

In the course of selection of the photometric system several photomultipliers were tested. The special image converter and optical system (designed in the laboratory) feeding converted wavelength into a blue sensitive photomultiplier proved impractical because it is most efficient when working from a system that presents a slit image on the image converter cathode. The Farnsworth PMI 15 converting stage photomultiplier had great sensitivity but the cathode noise fluctuations had more low frequency noise

than other systems. The RCA 7160 IR photomultiplier had the lowest noise in the working band and the best sensitivity when operated at full rating of 1800V without cooling the cathode. The advantage this tube enjoys is in large part determined by the fact that it can work from a diffuse image presented to the phototube. In addition to evaluating various infrared sensitive tubes for measurement of the 766 Potassium line, the 404 potassium line was tested with standard blue sensitive photomultipliers but sensitivity was about 1/20 of that of the 766 line. Spectra of the helium glow in the region of the 404 line were relatively free of interfering lines, so that it remains potentially useful.

Direction of Current Research:

The aim of the present research program is to provide a practical instrumental method for analysis of micropuncture samples for sodium and potassium.

Part B included - yes

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Part B

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Honors, Awards and Publications

Bowman, Robert L.: Helium Glow Discharge Spectroscopic Source for Microanalytic Determination of Alkali Metals, Digest of Technical Papers, 13th Annual Conference on Electrical Techniques in Medicine and Biology, page 90, Lewis Winner, Publisher, 1960.

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Serial No. NHI-311
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Development of a Program to Determine if Tissue Compatibility can be Predicted for Plastics in Synthetic Materials from Analysis of Physico-Chemical Properties

Principal Investigator: Robert L. Bowman

Other Investigators: John Ross

Cooperating Units: Clinical Surgery (Heart)
Battelle Memorial Institute (contract)

Man Years (calendar year 1960) Patient Days: None
Total: 0.3
Professional: 0.3

Project Description:

Progress During Past Year:

Tissue compatibility of synthetic materials for construction of prosthetic parts for the cardio-vascular system is now determined for a specific material as it becomes commercially available. These specific materials in their commercial form are so variable that it is difficult to compare results from the same material made by different suppliers. It is presumed that specific materials could be tailored to the tissue requirements if the desirable properties were defined in physico-chemical terms. This would make it possible for the modern technology of the plastics industry to provide materials with known surface and core properties. These technical achievements make it possible for several plastics to be grafted, one upon the other, to impart special surface properties on a core of more suitable mechanical properties. A discussion of some of these properties with the research staff of the Plastics Section of the Battelle Memorial Institute confirmed that they could produce special surface properties, special core properties and rather complex grafted materials. They also confirmed that they could impart acidic or basic surface properties, complete or partial cross-linking, or could protect exposed end groups with various chemical groups that would impart wettability, non-wettability or other specific surface properties. The

contract was negotiated to the extent of approximately twenty thousand dollars, and arrangements made for them to provide sample plastic teardrops, test tubes and other test pieces for physiological testing, to be carried on here in conjunction with specialized physical properties testing to be carried out at Battelle.

A number of plastic polymers were provided in highly purified form for physiological testing which was carried on by Dr. John Ross, Clinic of Surgery, while the physical properties were determined at Battelle. Details of this part of the project will be provided from the Clinic of Surgery. Results of the study indicated that the program was based on sound predications, but the duration of the program was too short to provide any long range information. It was felt that this type of program could be best handled through a grant of funds to a University Laboratory. When the material was presented at the Prosthetic Valve Conference in Chicago, it was determined that Dr. Kolff had already made some effort to organize a similar type of program. It was felt that Dr. Kolff's background in this field and extensive experience and qualifications as an investigator made him the ideal person to take over this project. The situation was discussed with Dr. Kolff and letters recommending that he apply for a grant for this project were sent to him. Dr. Kolff has indicated his enthusiastic reception of the idea that he continue this program and has already made arrangements with Battelle to continue the program.

Direction of Current Research:

No further activity in the development of this program is anticipated in this laboratory.

Part B included - yes

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Individual Project Report
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Part B Honors, Awards, and Publications

Ross, J., Greenfield, L. J., Bowman, R. L., Morrow, A. J.: The Chemical Structure and Surface Properties of Certain Synthetic Polymers and Their Relationship to Thrombosis, Proceedings of the Prosthetic Valve Conference, In press.

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